



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, C07K 14/47, C12N 15/62,</b> <b>C07K 16/18</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/21998</b>  <b>(43) International Publication Date:</b> 6 May 1999 (06.05.99)
<b>(21) International Application Number:</b> PCT/US98/22991  <b>(22) International Filing Date:</b> 29 October 1998 (29.10.98)  <b>(30) Priority Data:</b> 60/063,704                      29 October 1997 (29.10.97)                      US 60/073,612                      3 February 1998 (03.02.98)                      US 60/081,695                      14 April 1998 (14.04.98)                      US  <b>(71) Applicant:</b> GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).  <b>(72) Inventors:</b> BOTSTEIN, David, A.; 2539 Somerset Drive, Belmont, CA 94002 (US). COHEN, Robert, L.; 660 Parrott Drive, San Mateo, CA 94402 (US). GURNEY, Austin, L.; 1 Debbie Lane, Belmont, CA 94002 (US). HILLAN, Kenneth; 64 Seward Street, San Francisco, CA 94114 (US). LAWRENCE, David, A.; 1659 12th Avenue, San Francisco, CA 94122 (US). LEVINE, Arnold, J.; 138 FitzRandolph Road, Princeton, NJ 08540 (US). PENNICA, Diane; 2417 Hale Drive, Burlingame, CA 94010 (US). ROY, Margaret, Ann; 2960 Webster Street #4, San Francisco, CA 94123 (US). GODDARD, Audrey; 110 Congo Street, San Francisco, CA 94131 (US). WOOD, William, I.; 35 Southdown Court, Hillsborough, CA 94010 (US).		<b>(74) Agent:</b> BRUESS, Steven, C.; Merchant, Gould, Smith, Edell, Welter & Schmidt, P.A., 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> WNT-1 INDUCED SECRETED POLYPEPTIDES: WISP-1, -2 AND -3  <b>(57) Abstract</b>  Wnt-1-Induced Secreted Proteins (WISPs) are provided, whose genes are induced at least by Wnt-1. Also provided are nucleic acid molecules encoding those polypeptides, as well as vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides, and methods for producing the polypeptides.		

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This invention was made with government support under grant no. 5P01 CA41086, awarded by the National Institutes of Health, National Cancer Institute. The government has certain rights in the invention.

The present invention relates generally to the identification and isolation of novel DNA and to the recombinant production of novel polypeptides having homology to connective tissue growth factor, designated herein as **Wnt-1-Induced Secreted Proteins (WISPs)**.

Malignant tumors (cancers) are the second leading cause of death in the United States, after heart disease. Boring *et al.*, *CA Cancer J. Clin.*, 43: 7 (1993).

Alteration of gene expression is intimately related to the uncontrolled cell growth and differentiation which are a common feature of all cancers. The genomes of certain well studied tumors have been found to show decreased expression of recessive genes, usually referred to as tumor suppression genes, which would normally function to prevent malignant cell growth, and/or overexpression of certain dominant genes, such as oncogenes, that act to promote malignant growth. Each of these genetic changes appears to be responsible for importing some of the traits that, in aggregate, represent the full neoplastic phenotype. Hunter, *Cell*, 64: 1129 (1991); Bishop, *Cell*, 64: 235-248 (1991).

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. For example, it has been found that the human ErbB2 gene (*erbB2*, also known as *her2*, or *c-erbB-2*), which encodes a 185-kd transmembrane glycoprotein receptor (p185<sup>HER2</sup>; HER2) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer. Slamon *et al.*, Science, 235:177-182 (1987); Slamon *et al.*, Science, 244:707-712 (1989).

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Chromosomes Cancer, 1: 181-193 (1990); Alitalo *et al.*, *supra*. Thus, *erbB2* overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon *et al.*, (1987) and (1989), *supra*; Ravdin and Chamness, Gene, 159:19-27 (1995); and Hynes and Stern, Biochim Biophys Acta, 1198:165-184 (1994)), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines. Baselga *et al.*, Oncology, 11(3 Suppl 1):43-48 (1997). However, despite the association of *erbB2* overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients. Baselga *et al.*, *supra*. A recombinant humanized anti-ErbB2 (anti-HER2) monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or HERCEPTIN®) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anticancer therapy. Baselga *et al.*, J. Clin. Oncol., 14:737-744 (1996).

Cytokines have been implicated in the pathogenesis of a number of brain diseases in which neurological dysfunction has been attributed to a change in amino acid neurotransmitter metabolism. In particular, members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) family have been implicated. TGF peptides are small polypeptides that were first identified by their ability to induce proliferation and transformation in noncancerous cells in culture. Although initially defined as a growth factor, TGF- $\beta$  also inhibits proliferation of epithelial, endothelial, lymphoid, and hematopoietic cells. This cytokine is thought to play an important role in regulating the duration of the inflammatory response, allowing the healing process to proceed. It is also a potent immunomodulator, which has many pleiotrophic effects, including regulating many other cytokines.

The TGF- $\beta$  superfamily includes bone morphogenetic proteins (BMP-2, BMP-4, BMP-5, BMP-6, BMP-7), activins A & B, decapentaplegic (dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, Inhibin- $\alpha$ , TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 5, and glial-derived neurotrophic factor (GDNF). Atrisano *et al.*, J. Biochemica et Biophysica Acta, 1222:71-80 (1994). Of particular interest are the growth differentiation factors, for as their name implies, these factors are implicated in the differentiation of cells.

Connective tissue growth factor (CTGF) is a growth factor induced in fibroblasts by many factors, including TGF- $\beta$ , and is essential for the ability of TGF- $\beta$  to induce anchorage-independent growth (AIG), a property of transformed cells. CTGF was discovered in an attempt to identify the type of platelet-derived growth factor (PDGF) dimers present in the growth media of cultured endothelial cells, and is related immunologically and biologically to PDGF. See U.S. Pat. No. 5,408,040. CTGF also is mitogenic and chemotactic for cells, and hence growth factors in this family are believed to play a role in the normal development, growth, and repair of human tissue.

Seven proteins related to CTGF, including the chicken ortholog for Cyr61, CEF10, human, mouse, and *Xenopus laevis* CTGF, and human, chicken, and *Xenopus laevis* Nov have been isolated, cloned, sequenced, and characterized as belonging to the CTGF gene family. Oemar and Luescher, Arterioscler. Thromb. Vasc. Biol., 17: 1483-1489 (1997). The gene encoding Cyr61 has been found to promote angiogenesis, tumor growth, and vascularization. Babic *et al.*, Proc. Natl. Acad. Sci. USA, 95: 6355-6360 (1998). The *nov* gene is expressed in the kidney essentially at the embryonic stage, and alterations of *nov*



expression, relative to the normal kidney, have been detected in both avian nephroblastomas and human Wilms' tumors. Martinerie *et al.*, Oncogene, 9: 2729-2732 (1994). Wt1 downregulates human *nov* expression, which downregulation might represent a key element in normal and tumoral nephrogenesis. Martinerie *et al.*, Oncogene, 12: 1479-1492 (1996). It has recently been proposed that the CTGF, *nov*, and *cyr61* genes, which encode secreted proteins that contain conserved sequences and IGFBP motifs in their N-termini and bind IGFs with low affinity, represent more members of the IGFBP superfamily, along with the low-affinity mac25/IGFBP-7 (Yamanaka *et al.*, J. Biol. Chem., 272: 30729-30734 (1997)) and the high-affinity IGFBPs 1-6. CTGF under this proposal would be designated IGFBP-8. Kim *et al.*, Proc. Natl. Acad. Sci. USA, 94: 12981-12986 (1997).

Recently, a protein was found in the mouse designated ELM1 that is expressed in low metastatic cells. Hashimoto *et al.*, J. Exp. Med., 187: 289-296 (1998). The *elm1* gene, a mouse homologue of WISP-1 disclosed below, is another member of the CTGF. Cyr61/Cef10, and neuroblastoma overexpressed-gene family and suppresses *in vivo* tumor growth and metastasis of K-1735 murine melanoma cells. Another recent article on rCop-1, the rat orthologue of WISP-2 described below describes the loss of expression of this gene after cell transformation Zhang *et al.*, Mol. Cell. Biol., 18:6131-6141 (1998)

CTGF family members (with the exception of *nov*) are immediate early growth-responsive genes that are thought to regulate cell proliferation, differentiation, embryogenesis, and wound healing. Sequence homology among members of the CTGF gene family is high; however, functions of these proteins *in vitro* range from growth stimulatory (*i.e.*, human CTGF) to growth inhibitory (*i.e.*, chicken Nov and also possibly hCTGF). Further, some molecules homologous to CTGF are indicated to be useful in the prevention of desmoplasia, the formation of highly cellular, excessive connective tissue stroma associated with some cancers, and fibrotic lesions associated with various skin disorders such as scleroderma, keloid, eosinophilic fasciitis, nodular fasciitis, and Dupuytren's contracture. Moreover, CTGF expression has recently been demonstrated in the fibrous stroma of mammary tumors, suggesting cancer stroma formation involves the induction of similar fibroproliferative growth factors as wound repair. Human CTGF is also expressed at very high levels in advanced atherosclerotic lesions, but not in normal arteries, suggesting it may play a role in atherosclerosis. Oemar and Luescher, *supra*. Therefore, molecules homologous to CTGF are of importance.

Extracellular and membrane-bound proteins play important roles in the formation, differentiation, and maintenance of multicellular organisms. The fate of many individual cells, *e.g.*, proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones), which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment, usually at a membrane-bound receptor protein.

Secreted proteins have various industrial applications, including use as pharmaceuticals, diagnostics, biosensors, and bioreactors. In fact, most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secreted proteins. Their receptors, which are membrane-bound proteins, also have potential as therapeutic or

diagnostic agents. Receptor immunoadhesins, for instance, can be employed as therapeutic agents to block receptor-ligand interaction. Membrane-bound proteins can also be employed for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. Such membrane-bound proteins and cell receptors include, but are not limited to, cytokine receptors, receptor kinases, receptor phosphatases, receptors involved in cell-cell interactions, and cellular adhesion molecules like selectins and integrins. Transduction of signals that regulate cell growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine kinases, enzymes that catalyze that process, can also act as growth factor receptors. Examples include fibroblast growth factor receptor and nerve growth factor receptor.

Efforts are being undertaken by both industry and academia to identify new, native secreted and membrane-bound receptor proteins, particularly those having homology to CTGF. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted and membrane-bound receptor proteins. Examples of screening methods and techniques are described in the literature. See, for example, Klein *et al.*, Proc. Natl. Acad. Sci., 93:7108-7113 (1996); and U.S. Patent No. 5,536,637.

Wnts are encoded by a large gene family whose members have been found in round worms, insects, cartilaginous fish, and vertebrates. Holland *et al.*, Dev. Suppl., 125-133 (1994). Wnts are thought to function in a variety of developmental and physiological processes since many diverse species have multiple conserved *Wnt* genes. McMahon, Trends Genet., 8: 236-242 (1992); Nusse and Varmus, Cell, 69: 1073-1087 (1992). *Wnt* genes encode secreted glycoproteins that are thought to function as paracrine or autocrine signals active in several primitive cell types. McMahon, *supra* (1992); Nusse and Varmus, *supra* (1992). The *Wnt* growth factor family includes more than ten genes identified in the mouse (*Wnt*-1, -2, -3A, -3B, -4, -5A, -5B, -6, -7A, -7B, -8A, -8B, -10B, -11, -12, and -13) (see, e.g., Gavin *et al.*, Genes Dev., 4: 2319-2332 (1990); Lee *et al.*, Proc. Natl. Acad. Sci. USA, 92: 2268-2272 (1995); Christiansen *et al.*, Mech. Dev., 51: 341-350 (1995)) and at least nine genes identified in the human (*Wnt*-1, -2, -3, -5A, -7A, -7B, -8B, -10B, and -11) by cDNA cloning. See, e.g., Vant Veer *et al.*, Mol. Cell. Biol., 4: 2532-2534 (1984).

The *Wnt-1* proto-oncogene (*int-1*) was originally identified from mammary tumors induced by mouse mammary tumor virus (MMTV) due to an insertion of viral DNA sequence. Nusse and Varmus, Cell, 31: 99-109 (1982). In adult mice, the expression level of *Wnt-1* mRNA is detected only in the testis during later stages of sperm development. *Wnt-1* protein is about 42 KDa and contains an amino-terminal hydrophobic region, which may function as a signal sequence for secretion (Nusse and Varmus, *supra*, 1992). The expression of *Wnt-2/irp* is detected in mouse fetal and adult tissues and its distribution does not overlap with the expression pattern for *Wnt-1*. *Wnt-3* is associated with mouse mammary tumorigenesis. The expression of *Wnt-3* in mouse embryos is detected in the neural tubes and in the limb buds. *Wnt-5a* transcripts are detected in the developing fore- and hind limbs at 9.5 through 14.5 days and highest levels are concentrated in apical ectoderm at the distal tip of limbs. Nusse and Varmus, *supra* (1992). Recently, a *Wnt* growth factor, termed *Wnt-x*, was described (WO95/17416) along with the detection of *Wnt-x* expression in bone tissues and in bone-derived cells. Also described was the role of *Wnt-x* in the maintenance of mature

osteoblasts and the use of the Wnt-x growth factor as a therapeutic agent or in the development of other therapeutic agents to treat bone-related diseases.

Wnts may play a role in local cell signaling. Biochemical studies have shown that much of the secreted Wnt protein can be found associated with the cell surface or extracellular matrix rather than freely diffusible in the medium. Papkoff and Schryver, Mol. Cell. Biol., 10: 2723-2730 (1990); Bradley and Brown, EMBO J., 9: 1569-1575 (1990).

Studies of mutations in *Wnt* genes have indicated a role for *Wnts* in growth control and tissue patterning. In *Drosophila*, *wingless* (*wg*) encodes a *Wnt*-related gene (Rijsewijk *et al.*, Cell, 50: 649-657 (1987)) and *wg* mutations alter the pattern of embryonic ectoderm, neurogenesis, and imaginal disc outgrowth. Morata and Lawrence, Dev. Biol., 56: 227-240 (1977); Baker, Dev. Biol., 125: 96-108 (1988); Klingensmith and Nusse, Dev. Biol., 166: 396-414 (1994). In *Cuenorhabditis elegans*, *lin-44* encodes a Wnt homolog which is required for asymmetric cell divisions. Herman and Horvitz, Development, 120: 1035-1047 (1994). Knock-out mutations in mice have shown Wnts to be essential for brain development (McMahon and Bradley, Cell, 62: 1073-1085 (1990); Thomas and Cappechi, Nature, 346: 847-850 (1990)), and the outgrowth of embryonic primordia for kidney (Stark *et al.*, Nature, 372: 679-683 (1994)), tail bud (Takada *et al.*, Genes Dev., 8: 174-189 (1994)), and limb bud. Parr and McMahon, Nature, 374: 350-353 (1995). Overexpression of *Wnts* in the mammary gland can result in mammary hyperplasia (McMahon, *supra* (1992); Nusse and Varmus, *supra* (1992)), and precocious alveolar development. Bradbury *et al.*, Dev. Biol., 170: 553-563 (1995).

*Wnt-5a* and *Wnt-5b* are expressed in the posterior and lateral mesoderm and the extraembryonic mesoderm of the day 7-8 murine embryo. Gavin *et al.*, *supra* (1990). These embryonic domains contribute to the AGM region and yolk sac tissues from which multipotent hematopoietic precursors and HSCs are derived. Dzierzak and Medvinsky, Trends Genet., 11: 359-366 (1995); Zon *et al.*, in Gluckman and Coulombel, ed., Colloque, INSERM, 235: 17-22 (1995), presented at the Joint International Workshop on Foetal and Neonatal Hematopoiesis and Mechanism of Bone Marrow Failure, Paris France, April 3-6, 1995; Kanatsu and Nishikawa, Development, 122: 823-830 (1996). *Wnt-5a*, *Wnt-10b*, and other *Wnts* have been detected in limb buds, indicating possible roles in the development and patterning of the early bone microenvironment as shown for *Wnt-7b*. Gavin *et al.*, *supra* (1990); Christiansen *et al.*, Mech. Devel., 51: 341-350 (1995); Parr and McMahon, *supra* (1995).

The Wnt/Wg signal transduction pathway plays an important role in the biological development of the organism and has been implicated in several human cancers. This pathway also includes the tumor suppressor gene, APC. Mutations in the APC gene are associated with the development of sporadic and inherited forms of human colorectal cancer. The Wnt/Wg signal leads to the accumulation of beta-catenin/Armadillo in the cell, resulting in the formation of a bipartite transcription complex consisting of beta-catenin and a member of the lymphoid enhancer binding factor/T cell factor (LEF/TCF) HMG box transcription factor family. This complex translocates to the nucleus where it can activate expression of genes downstream of the Wnt/Wg signal, such as the engrailed and Ultrabithorax genes in *Drosophila*. The downstream target genes of Wnt-1 signaling in vertebrates that presumably function in tumorigenesis, however, are currently unknown.

For a most recent review on Wnt, see Cadigan and Nusse, Genes & Dev., 11: 3286-3305 (1997).

There is a need to elucidate the further members of the above families, including cell-surface molecules that may be tumor-specific antigens or proteins that serve a regulatory function in initiating the Wnt pathway of tumorigenesis. These would also include downstream components of the Wnt signaling pathway that are important to the transformed phenotype and the development of cancer.

#### SUMMARY OF THE INVENTION

Several putative Wnt-1-induced genes have been identified at the mRNA level in a high-throughput cDNA subtraction experiment. Thus, applicants have identified novel cDNA clones (*WISP1*, *WISP2*, and *WISP3*) that encode novel polypeptides of the WISP family, designated as WISP-1, WISP-2, and WISP-3, respectively. This class of polypeptides was formerly referred to as Wnt-1-Induced Gene (WIG) polypeptides, with WISP-1 and WISP-2 formerly designated as WIG-1 and WIG-2, respectively. One of the cDNA clones encodes a novel polypeptide, human WISP-2, having homology to CTGF, wherein the polypeptide is designated in the present application as "human WISP-2" or "PRO261". The WISP-1 and WISP-3 molecules also have homology to CTGF.

In one embodiment, this invention provides isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid has at least one WISP biological activity. In a more preferred embodiment, this nucleic acid has at least about a 95% sequence identity to (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO: 3), or (b) the complement of the DNA molecule of (a).

More preferred is the nucleic acid comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or DNA encoding a human WISP-1 polypeptide having amino acid residues 1 to 367 of Figures 3A and 3B (SEQ ID NO:4), or the complement of either of the encoding DNAs. Further preferred is this nucleic acid comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 or 1 to 367 of Figures 3A and 3B except for an isoleucine residue at position 184 rather than a valine residue or a serine residue at position 202 rather than an alanine residue (SEQ ID NOS:5-8, respectively). Further preferred also is this nucleic acid comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 or 1 to 367 of Figures 3A and 3B except for an isoleucine residue at position 184 rather than a valine residue and a serine residue at position 202 rather than an alanine residue (SEQ ID NOS:21-22, respectively).

Also preferred is this nucleic acid comprising DNA encoding a mouse WISP-1 polypeptide having amino acid residues 23 to 367 of Figure 1 (SEQ ID NO:11), or DNA encoding a mouse WISP-1 polypeptide having amino acid residues 1 to 367 of Figure 1 (SEQ ID NO:12), or the complement of either of the encoding DNAs.

Also provided by this invention is isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 85% sequence identity to (a) a DNA molecule encoding a mouse WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figure 1 (SEQ ID NO:11), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid has at least one WISP biological

activity. More preferably, this nucleic acid comprises DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a mouse WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figure 1 (SEQ ID NO:11), or (b) the complement of the DNA molecule of (a).

5 In another preferred embodiment, the invention provides an isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-1 polypeptide cDNA in ATCC Deposit No. 209533 (pRK5E.h.WISP-1.568.38), or (b) the complement of the DNA molecule of (a). This nucleic acid preferably comprises DNA having at least about 600 nucleotides and at least about a 95% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human  
10 WISP-1 polypeptide cDNA in ATCC Deposit No. 209533 (pRK5E.h.WISP-1.568.38) or (b) the complement of the DNA molecule of (a).

In another aspect, the invention provides a process for producing a WISP-1 polypeptide comprising culturing a host cell comprising the above nucleic acid under conditions suitable for expression of the WISP-1 polypeptide and recovering the WISP-1 polypeptide from the cell culture. Additionally provided is an  
15 isolated WISP-1 polypeptide encoded by the above nucleic acid, including where the polypeptide is human WISP-1 or mouse WISP-1.

In another embodiment, the invention provides isolated nucleic acid comprising SEQ ID NO:23, 24, 25, 26, 27, 28, or 29, and an isolated WISP-1 polypeptide encoded by such a nucleic acid.

Also provided by this invention is an isolated nucleic acid having at least about 600 nucleotides and  
20 produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a), and, if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), isolating the test DNA molecule.

Further provided is a polypeptide produced by (i) hybridizing a test DNA molecule under stringent  
25 conditions with (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

30 In another aspect, the invention provides isolated nucleic acid comprising DNA having at least about an 80% sequence identity to (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid has at least one WISP biological activity. Also, preferably this nucleic acid comprises DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a human  
35 WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) the complement of the DNA molecule of (a). In another preferred embodiment, this nucleic acid comprises DNA encoding a human WISP-2 polypeptide having amino acid residues 24 to 250 of Figure 4 (SEQ ID NO:15), or DNA encoding a human WISP-2 polypeptide having amino acid residues 1 to 250 of Figure 4 (SEQ ID NO:16), or a complement of either of the encoding DNAs.

In another aspect, the invention provides isolated nucleic acid comprising DNA having at least about an 80% sequence identity to (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 1 to 250 of Figure 4 (SEQ ID NO:16), or (b) the complement of the DNA molecule of (a).

5 In another aspect, the invention provides isolated nucleic acid comprising DNA having at least about 500 nucleotides and at least about an 80% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 24 to 251 of Figure 2 (SEQ ID NO:19), or (b) the complement of the DNA molecule of (a). In a preferred embodiment, this nucleic acid comprises DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 24 to 251 of Figure 2 (SEQ ID NO:19), or (b) the complement of the DNA molecule of (a). More preferably, the nucleic acid comprises DNA encoding a mouse WISP-2 polypeptide having amino acid residues 24 to 251 of Figure 2 (SEQ ID NO:19), or DNA encoding a mouse WISP-2 polypeptide having amino acid residues 1 to 251 of Figure 2 (SEQ ID NO:20), or the complement of either of these encoding DNAs.

15 In a further aspect, the invention provides isolated nucleic acid comprising DNA having at least about 500 nucleotides and at least about an 80% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 1 to 251 of Figure 2 (SEQ ID NO:20), or (b) the complement of the DNA molecule of (a).

20 In yet another aspect, the invention provides an isolated nucleic acid comprising DNA having at least about 400 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-2 polypeptide cDNA in ATCC Deposit No. 209391 (DNA33473), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid comprises DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-2 polypeptide cDNA in ATCC Deposit No. 209391 (DNA33473), or (b) the complement of the DNA molecule of (a).

25 In another embodiment, this invention provides an isolated nucleic acid comprising the nucleotide sequence of the full-length coding sequence of clone UNQ228 (DNA33473) deposited under accession number ATCC 209391.

30 In another aspect, the invention provides a process for producing a WISP-2 polypeptide comprising culturing a host cell comprising the above nucleic acid under conditions suitable for expression of the WISP-2 polypeptide and recovering the WISP-2 polypeptide from the cell culture. Additionally provided is a WISP-2 polypeptide encoded by the isolated nucleic acid, including where the polypeptide is human WISP-2 or mouse WISP-2. In a specific embodiment of this, the invention provides isolated native-sequence human WISP-2 polypeptide comprising amino acid residues 1 to 250 of Figure 4 (SEQ ID NO:16) or comprising amino acid residues 24 to 250 of Figure 4 (SEQ ID NO:15).

35 In a further embodiment, the invention provides an isolated nucleic acid having at least about 400 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure

4 (SEQ ID NO:15), or (b) the complement of the DNA molecule of (a), and, if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), isolating the test DNA molecule.

In a still further embodiment, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In yet another embodiment, the invention provides isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 500 nucleotides to (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid has at least one WISP biological activity. Preferably, this nucleic acid comprises DNA encoding a human WISP-3 polypeptide having amino acid residues 34 to 372 of Figures 6A and 6B (SEQ ID NO:32) or amino acids 1 to 372 of Figures 6A and 6B (SEQ ID NO:33), or the complement thereof.

In a still further embodiment, the invention provides an isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 500 nucleotides to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-3 polypeptide cDNA in ATCC Deposit No. 209706 (DNA56350-1176-2), or (b) the complement of the DNA molecule of (a). A still further aspect of the invention involves a process for producing a WISP-3 polypeptide comprising culturing a host cell comprising WISP-3-encoding nucleic acid under conditions suitable for expression of the WISP-3 polypeptide and recovering the WISP-3 polypeptide from the cell culture.

Further provided is an isolated WISP-3 polypeptide encoded by the WISP-3-encoding nucleic acid. Preferably, this polypeptide is human WISP-3.

In another embodiment, the invention provides an isolated nucleic acid produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) the complement of the DNA molecule of (a), and, if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 500 nucleotides, isolating the test DNA molecule.

Also provided is a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 500 nucleotides, (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In yet another embodiment, the invention provides isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 400 nucleotides to (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) the complement of the DNA molecule of (a). Preferably, this nucleic acid has at least one

WISP biological activity. Preferably, this nucleic acid comprises DNA encoding a human WISP-3 polypeptide having amino acid residues 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or amino acid residues 1 to 355 of Figures 7A and 7B (SEQ ID NO:37) or the complement thereof.

5 In a still further embodiment, the invention provides an isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 400 nucleotides to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-3 polypeptide cDNA in ATCC Deposit No. 209707 (DNA58800-1176-2), or (b) the complement of the DNA molecule of (a).

10 A still further aspect of the invention involves a process for producing a WISP-3 polypeptide of Fig. 7A and 7B comprising culturing a host cell comprising WISP-3-encoding nucleic acid under conditions suitable for expression of the WISP-3 polypeptide and recovering the WISP-3 polypeptide from the cell culture.

Further provided is an isolated WISP-3 polypeptide of Fig. 7A and 7B encoded by the WISP-3-encoding nucleic acid. Preferably, this polypeptide is human WISP-3.

15 In another embodiment, the invention provides an isolated nucleic acid produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) the complement of the DNA molecule of (a), and, if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 400 nucleotides, isolating the test DNA molecule.

20 Also provided is a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 400 nucleotides, (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

25 Preferably the complements of the DNA molecules herein remain stably bound to the primary sequence under at least moderate, and optionally, under high stringency conditions.

Also provided are vectors comprising the above nucleic acids, host cells comprising the vector, preferably wherein the cell is a Chinese hamster ovary (CHO) cell, an *E. coli* cell, a baculovirus-infected cell, or a yeast cell.

30 Additionally provided are a chimeric molecule comprising one of the above polypeptides or an inactivated variant thereof, fused to a heterologous amino acid sequence, wherein the heterologous amino acid sequence may be, for example, an epitope tag sequence, a polyamino acid such as poly-histidine, or an immunoglobulin constant region (Fc). Also provided is an antibody which specifically binds to one of the above polypeptides, wherein the antibody can be a monoclonal antibody.

35 Further provided are a composition comprising one of the above polypeptides and a carrier therefor, and a composition comprising an antagonist to one of the polypeptides and a carrier therefor. In one such embodiment, the invention provides a composition comprising a WISP-1, WISP-2, or WISP-3 polypeptide and a pharmaceutically acceptable carrier. Preferably, the polypeptide is a human polypeptide. Also, preferably, these compositions may also comprise a chemotherapeutic agent or growth-inhibitory agent.



In another aspect, the invention provides a pharmaceutical product comprising:

(a) the composition comprising WISP-1, WISP-2, or WISP-3 polypeptide and a pharmaceutically acceptable carrier;

(b) a container containing said composition; and

5 (c) a label affixed to said container, or a package insert included in said pharmaceutical product referring to the use of said WISP-1, WISP-2, or WISP-3 polypeptide in the treatment of a WISP-related disorder.

In yet another embodiment, the invention provides a method for treating a WISP-related disorder in a mammal comprising administering to the mammal an effective amount of any of the above compositions, including the composition of a WISP-1, WISP-2, or WISP-3 polypeptide in a pharmaceutically acceptable carrier, and including the composition of an antagonist to a WISP-1, WISP-2, or WISP-3 polypeptide in a pharmaceutically acceptable carrier. Preferably, the disorder is a malignant disorder or arteriosclerosis. More preferably, the malignant disorder is breast cancer, ovarian cancer, colon cancer, or melanoma. Also, preferably the mammal is human. In another preferred embodiment, the WISP-1, WISP-2, or WISP-3 polypeptide is administered in combination with a chemotherapeutic agent, a growth inhibitory agent, or a cytotoxic agent.

In another embodiment, the invention supplies a process for diagnosing a disease or a susceptibility to a disease related to a mutation in a nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide comprising:

20 (a) isolating a nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide from a sample derived from a host; and

(b) determining a mutation in the nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide.

In another embodiment, the invention provides a method of diagnosing a WISP-related disorder in a mammal comprising detecting the level of expression of a gene encoding a WISP-1, WISP-2, or WISP-3 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample indicates the presence of a WISP-related dysfunction in the mammal from which the test tissue cells were obtained. Preferably, such a disorder is a type of cancer and a higher expression level in the test sample indicates the presence of a tumor in the mammal.

30 In a still further embodiment, the invention provides an isolated antibody binding a WISP-1, WISP-2, or WISP-3 polypeptide. Preferably, the antibody induces death of a cell overexpressing a WISP-1, WISP-2, or WISP-3 polypeptide, more preferably a cancer cell. Also preferred is an antibody that binds to a human WISP-1, WISP-2, or WISP-3 polypeptide, and is a human or humanized antibody. More preferred is a monoclonal antibody, still more preferred, a monoclonal antibody that has complementary-determining regions and constant immunoglobulin regions, and in other embodiments is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody. In addition, the antibody is suitably labeled with a detectable label or immobilized on a solid support.

Also provided is a composition comprising an antibody to a WISP-1, WISP-2, or WISP-3 polypeptide in admixture with a pharmaceutically acceptable carrier. Preferably, the antibody is to a human WISP-1, WISP-2, or WISP-3 polypeptide, and is a human or humanized antibody, most preferably a monoclonal antibody against human WISP-1. Further, the composition may comprise a growth-inhibitory amount of said antibody.

In another embodiment, the invention provides a method for treating cancer in a mammal comprising administering to the mammal an effective amount of the above antibody composition. In a preferred aspect of this method, the cancer is colon cancer, the antibody is against human WISP-1 and is a humanized or human monoclonal antibody, and the mammal is human.

In another aspect, the invention provides a method for treating a WISP-related disorder in a mammal comprising administering to the mammal an effective amount of a composition comprising an antagonist to a WISP-1, WISP-2, or WISP-3 polypeptide in a pharmaceutically acceptable carrier.

In a further aspect, the invention provides a method for inhibiting the growth of tumor cells comprising exposing a cell that overexpresses a Wnt-1-induced gene to an effective amount of an antagonist that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide.

A further aspect entails a method for inhibiting the growth of tumor cells comprising exposing said cells to an effective amount of the composition with the growth-inhibiting amount of an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody in admixture with the carrier. In a preferred aspect of this method, the tumor cells are colon cancer cells, the antibody is against human WISP-1 and is a humanized or human monoclonal antibody, and the mammal is human.

Also provided herein is a kit comprising one of the above WISP polypeptides or WISP antagonists, such as anti-WISP antibodies, and instructions for using the polypeptide or antagonist to detect or treat a WISP-related disorder, such as cancer induced by Wnt. One such preferred kit is a cancer diagnostic kit comprising an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody and a carrier in suitable packaging. Preferably, this kit further comprises instructions for using said antibody to detect the WISP-1, WISP-2, or WISP-3 polypeptide.

Also provided is a method for inducing cell death comprising exposing a cell which is induced by Wnt to an effective amount of one of the above WISP polypeptides or WISP antagonists, such as anti-WISP antibodies. Preferably, such cell is a cancer cell. More preferably, the cell is in a mammal, more preferably a human. In addition, an effective amount of another chemotherapeutic antibody is used in the exposure of the cell, such as an anti-ErbB2 antibody. Further, optionally the method comprises exposing the cell to a chemotherapeutic agent, a growth-inhibitory agent, or radiation. Optionally, the cell is exposed to the growth-inhibitory agent prior to exposure to the antibody.

In a further aspect, the invention provides an article of manufacture, comprising:

a container;

a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inducing cell death or inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overinduction of Wnt or a WISP-

related disorder or by overexpression of a WISP-1, WISP-2, or WISP-3 polypeptide, and the active agent in the composition is an antagonist to one of the polypeptides, that is, an agent that inhibits the expression and/or activity of the WISP-1, WISP-2, or WISP-3 polypeptide. Preferably, the active agent in such article of manufacture is an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody, and the label on the container indicates that the composition can be used for treating a WISP-related disorder.

In another embodiment, the invention provides a process for identifying agonists to a WISP-1, WISP-2, or WISP-3 polypeptide comprising:

(a) contacting cells and a compound to be screened under conditions suitable for the stimulation of cell proliferation by the polypeptide; and

(b) measuring the proliferation of the cells to determine if the compound is an effective agonist.

Additionally, the invention provides an agonist to a WISP-1, WISP-2, or WISP-3 polypeptide identified by the above process.

Further, the invention provides a method for identifying a compound that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide, comprising contacting a candidate compound with a WISP-1, WISP-2, or WISP-3 polypeptide under conditions and for a time sufficient to allow the compound and polypeptide to interact. In a preferred embodiment, this method comprises the steps of:

(a) contacting cells and a compound to be screened in the presence of the WISP-1, WISP-2, or WISP-3 polypeptide under conditions suitable for the stimulation of cell proliferation by polypeptide; and

(b) measuring the proliferation of the cells to determine if the compound is an effective antagonist.

Further, a compound identified by this method is provided.

In another aspect, this invention provides a compound that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide.

In another embodiment, the invention provides a method for determining the presence of a WISP-1, WISP-2, or WISP-3 polypeptide comprising exposing a cell suspected of containing the WISP-1, WISP-2, or WISP-3 polypeptide to an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody and determining binding of said antibody to said cell.

In another preferred embodiment, the invention provides a method of diagnosing a WISP-related disorder in a mammal comprising (a) contacting an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody and the WISP-1, WISP-2, or WISP-3 polypeptide in the test sample. Preferably, said test sample is obtained from an individual suspected to have neoplastic cell growth or proliferation. Also, preferably the antibody is labeled with a detectable label and/or is immobilized on a solid support.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the derived amino acid sequence of a native-sequence mouse WISP-1 protein from amino acids 1 to 367 (SEQ ID NO:12) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:9 and 10, respectively). There is a 1104-bp coding region and 584 bp of 3' untranslated region. In the Figure, amino acids 1 through 22 form a putative signal sequence, amino acids 23 through 367 are the putative mature protein (SEQ ID NO:11), with amino acids 86 to 88, 143 to 145, 284

to 286, and 343 to 345 being potential glycosylation sites. Potential protein kinase C phosphorylation sites are at amino acids 43-45, 159-161, 235-237, 292-294, 295-297, and 345-347. Potential casein kinase II phosphorylation sites are at amino acids 44-47, 131-134, 145-148, and 358-361. Potential N-myristoylation sites are at amino acids 18-23, 72-77, 127-132, 149-154, 231-236, and 289-294. A potential amidation site is at amino acids 269-272. A potential prokaryotic membrane lipoprotein lipid attachment site is at amino acids 113-123. A potential von Willebrand C1 domain is at amino acids 130-146. A potential thrombospondin I domain is at amino acids 223-237. A potential CT module is at amino acids 301-312. A potential IGF binding protein consensus site is at amino acids 72-80.

Figure 2 shows the derived amino acid sequence of a native-sequence mouse WISP-2 protein from amino acids 1 to 251 (SEQ ID NO:20) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:17 and 18, respectively) from a clone 1367.3. There are 756 bp of coding nucleotides and 722 bp of 3' untranslated region. In the Figure, amino acids 1 through 23 form a putative signal sequence; amino acids 24 through 251 are the putative mature protein (SEQ ID NO:19). A potential N-glycosylation site is at amino acids 197-200. A potential glycosaminoglycan attachment site is at amino acids 85-88. Potential protein kinase C phosphorylation sites are at amino acids 85-87 and 112-114. Potential N-myristoylation sites are at amino acids 49-54, 81-86, 126-131, 210-215, and 245-250. A potential amidation site is at amino acids 103-106. A potential phospholipase A2 aspartic acid active site is at amino acids 120-130. A potential IGF binding protein consensus signature is at amino acids 49-64. A potential von Willebrand C1 domain is at amino acids 107-123. A potential thrombospondin I domain is at amino acids 202-216. A potential IGF binding protein consensus site is at amino acids 49-57.

Figures 3A and 3B show the derived amino acid sequence of a native-sequence human WISP-1 protein from amino acids 1 to 367 (SEQ ID NO:4) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:1 and 2, respectively). There are 1104 bp of coding region in this human clone 568.38, and 1638 bp of 3' untranslated region. In the Figure, amino acids 1 through 22 form a putative signal sequence; amino acids 23 through 367 are the putative mature protein (SEQ ID NO:3), with amino acids 85 to 87, 143 to 145, 284 to 286, and 343 to 345 being potential glycosylation sites. A potential cAMP- and cGMP-dependent protein kinase phosphorylation site is from amino acids 171 to 174; potential protein kinase C phosphorylation sites are at amino acids 43-45, 235-237, 292-294, and 345-347. Potential casein kinase II phosphorylation sites are at amino acids 30-33, 145-148, and 358-361. Potential N-myristoylation sites are at amino acids 72-77, 127-132, 149-154, 201-206, 231-236, 289-294, and 327-332. A potential amidation site is at amino acids 269-272. A potential prokaryotic membrane lipoprotein lipid attachment site is at amino acids 113-123. A potential von Willebrand C1 domain is at amino acids 130-146. A potential thrombospondin I domain is at amino acids 223-237. A potential CT (C-Terminal) module is at amino acids 301-312. A potential IGF binding protein consensus site is at amino acids 72-80.

Figure 4 shows the derived amino acid sequence of a native-sequence human WISP-2 protein from amino acids 1 to 250 (SEQ ID NO:16) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:13 and 14, respectively). The coding region is 753 bp and the 3' untranslated region is 519 bp. The putative signal sequence is from amino acid residues 1 through 23 and the putative mature region is from 24 through 250 (SEQ ID NO:15). The clone designated herein as "UNQ228" and/or

"DNA33473-seqmin" (SEQ ID NO:38) begins at nucleotide 13 of SEQ ID NO:13. Potential protein kinase C phosphorylation sites are at amino acids 4-6, 118-120, and 227-229. A potential casein kinase II phosphorylation site is at amino acids 98-101. A potential N-myristoylation site is at amino acids 3-8, 49-54, 81-86, 85-90, 126-131, 164-169, 166-171, 167-172, 183-188, and 209-214. A potential IGF binding protein consensus signature is at amino acids 49-64. A potential von Willebrand C1 domain is at amino acids 107-123. A potential thrombospondin I domain is at amino acids 201-215. A potential IGF binding protein consensus site is at amino acids 49-57.

Figure 5 shows a 841-bp consensus nucleotide sequence designated "DNA30843" (SEQ ID NO:39) derived from the nucleotide sequences of twenty different expressed sequence tags from Incyte. When aligned with the other sequences, DNA30843 has 3 gaps. It has 441 bp orf (+1). DNA30843 was used to design probes for isolation of human WISP-2.

Figures 6A and 6B show the derived amino acid sequence of a native-sequence human WISP-3 protein from amino acids 1 to 372 (SEQ ID NO:33) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:30 and 31, respectively). In the Figure, amino acids 1 through 33 form a putative signal sequence, amino acids 34 through 372 are the putative mature protein (SEQ ID NO:32), with amino acids 196 to 198 and 326 to 328 being potential glycosylation sites. Potential protein kinase C phosphorylation sites are at amino acids 209-211, 246-248, 277-279, 308-310, and 342-344. Potential casein kinase II phosphorylation sites are at amino acids 47-50, 254-257, and 293-296. Potential N-myristoylation sites are at amino acids 21-26, 89-94, 139-144, 166-171, 180-185, 185-190, 188-193, 242-247, and 302-307. A potential amidation site is at amino acids 188-191. Potential prokaryotic membrane lipoprotein lipid attachment sites are at amino acids 130-140 and 160-170. A potential IGF binding protein signature site is at amino acids 89-104. A potential IGF binding protein site (less stringent than prosite's) is at amino acids 89-97.

Figures 7A and 7B show the derived amino acid sequence of a native-sequence human WISP-3 protein from amino acids 1 to 355 (SEQ ID NO:37) and the nucleotide sequence (and complementary sequence) encoding the protein (SEQ ID NOS:34 and 35, respectively). This protein is believed to be a splice variant of the nucleotide sequence shown in Figure 6 with a shorter 5' end. In the Figure, amino acids 1 through 15 form a putative signal sequence, amino acids 16 through 355 are the putative mature protein (SEQ ID NO:36), with amino acids 178 to 180 and 308 to 310 being potential glycosylation sites. Potential protein kinase C phosphorylation sites are at amino acids 191-193, 228-230, 259-261, 290-292, and 324-326. Potential casein kinase II phosphorylation sites are at amino acids 29-32, 236-239, and 275-278. Potential N-myristoylation sites are at amino acids 3-8, 71-76, 121-126, 148-153, 162-167, 167-172, 170-175, 224-229, and 284-289. A potential amidation site is at amino acids 170-173. Potential prokaryotic membrane lipoprotein lipid attachment sites are at amino acids 112-122 and 142-152. A potential IGF binding protein signature site is at amino acids 71-87. A potential IGF binding protein site (less stringent than prosite's) is at amino acids 71-79.

Figure 8 shows an alignment of the full-length amino acid sequences of the human and mouse WISP-1 (SEQ ID NOS:4 and 12, respectively).

Figure 9 shows an alignment of the full-length amino acid sequences of the human and mouse WISP-2 (SEQ ID NOS:16 and 20, respectively).

Figure 10 shows an alignment of the amino acid sequences of the two clones of human WISP-3.

Figures 11A-11C show an alignment of the nucleotide sequences of human WISP-1 and the human WISP-3 shown in Fig. 6.

Figure 12 shows an alignment of the amino acid sequences of human WISP-1 and the human WISP-3 shown in Fig. 6.

Figure 13 shows a map of the vector pBabe puro (5.1 kb) used to transform cells for purposes of differential expression. The vector includes both unique restriction sites and multiple restriction sites. It is shown here in modified form for Wnt-1 cloning wherein the *HindIII* site after the SV40 promoter in the original pBabe puro vector has been removed and a *HindIII* site added to the multiple cloning site of the original pBabe puro vector. Wnt-1 is cloned from *EcoRI-HindIII* in the multiple cloning site. Constructs derived from this vector are selected in ampicillin (100 µg/ml) and the cells infected in culture are selected in 1.0-2.5 µg/ml puromycin.

Figure 14 shows the sequences of the PCR-Select® cDNA synthesis primer (SEQ ID NO:40), adaptors 1 and 2 (SEQ ID NOS:41 and 42, respectively) and complementary sequences for the adaptors (SEQ ID NOS:43 and 44, respectively). PCR primer 1 (SEQ ID NO:45), PCR primer 2 (SEQ ID NO:46), nested PCR primer 1 (SEQ ID NO:47), nested PCR primer 2 (SEQ ID NO:48), control primer G3PDH 5' primer (SEQ ID NO:49), and control primer G3PDH 3' primer (SEQ ID NO:50) used for suppression subtractive hybridization for identifying WISP clones. When the adaptors are ligated to *RsaI*-digested cDNA, the *RsaI* site is restored.

Figure 15 shows the cloning site region of the plasmid pGEM-T used to clone all of the WISP sequences herein (SEQ ID NOS:51 and 52 for 5' and 3' sequences, respectively).

Figures 16A-16D show the sequence (SEQ ID NO:53) of a plasmid that is used to prepare an expression plasmid for expression of mouse WISP-1 in mammalian cells, the latter being designated pRK5.CMV.puro-dhfr.mWISP-1.6His.

Figures 17A-17D show the sequence (SEQ ID NO:54) of plasmid pb.PH.IgG, which is used to prepare an expression plasmid for expression of mouse WISP-1 DNA in baculovirus-infected insect cells.

Figures 18A-18D show the sequence (SEQ ID NO:55) of plasmid pbPH.His.c, which is used to prepare an expression plasmid for expression of mouse WISP-1 DNA in baculovirus-infected insect cells, the latter being designated pbPH.mu.568.8his.baculo.

Figures 19A-19D show graphs of the delta CT in nine colon cancer cell lines and DNA from the blood of ten normal human donors (Nor Hu) as control, for human TNF, human WISP-1, Lyra, and human Apo2 ligand, respectively, using the ABI Prism 7700™ Sequence Detection System procedure for testing genomic amplification.

Figures 20A-20D show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control, for human DCR1, huFAS, human WISP-2, and Apo3, respectively, using the ABI Prism 7700™ Sequence Detection System procedure for testing genomic amplification.

Figures 21A-21D show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control for three different runs of human WISP-1 (designated in the figure as huWISP-1c, -1b, and -1a) and the average of these three runs of human WISP-1, respectively, using the ABI Prism 7700<sup>TM</sup> Sequence Detection System procedure for testing genomic amplification.

Figures 22A-22D show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control for three different runs of human WISP-2 (designated in the figure as huWISP-2c, -2b, and -2a; Figs. 22A, C, and D, respectively) and the average of these three runs of human WISP-2 (Fig. 22B), using the ABI Prism 7700<sup>TM</sup> Sequence Detection System procedure for testing genomic amplification.

Figures 23A-23C show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control for two different runs of human DR5 (DR5a and DR5b) and the average of these two runs of DR5, respectively, using the ABI Prism 7700<sup>TM</sup> Sequence Detection System procedure for testing genomic amplification.

Figures 24A-24D show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control for four different runs of *c-myc* (*c-myc*(a1), *c-myc*(b1), *c-myc*(b), and *c-myc*(a)), respectively, using the ABI Prism 7700<sup>TM</sup> Sequence Detection System procedure for testing genomic amplification.

Figures 25A-25D show graphs of the delta CT in nine colon cancer cell lines and Nor Hu as control for two different runs of human WISP-1 (designated in the figure as huWISP-1(a) and huWISP-1(b)) and for two different runs of human WISP-2 (designated in the figure as huWISP-2(a) and huWISP-2(b)), respectively, using the ABI Prism 7700<sup>TM</sup> Sequence Detection System procedure for testing genomic amplification.

Figure 26 shows the sequence (SEQ ID NO:23) of clone 568.13, a potential splice variant of human WISP-1 obtained by screening with a probe derived from clone 568.15A, which is the initial clone isolated from a human lung library in the process to obtain full-length human WISP-1 DNA.

Figure 27 shows the sequence (SEQ ID NO:24) of clone 568.1A, a potential human WISP-1 splice variant, 5' end only, obtained by screening with a probe derived from clone 568.15A.

Figure 28 shows the sequence (SEQ ID NO:25) of clone 568.39, a potential human WISP-1 splice variant, 5' end only, obtained by screening with a probe derived from clone 568.15A.

Figure 29 shows the sequence (SEQ ID NO:26) of clone 568.4A, a potential human WISP-1 splice variant obtained by screening with a probe derived from clone 568.15A.

Figure 30 shows the sequence (SEQ ID NO:27) of clone 568.5A, a potential human WISP-1 splice variant, 5' end only, obtained by screening with a probe derived from clone 568.15A.

Figure 31 shows the sequence (SEQ ID NO:28) of clone 568.6B, a potential human WISP-1 splice variant, 5' end only, obtained by screening with a probe derived from clone 568.15A.

Figure 32 shows the sequence (SEQ ID NO:29) of clone 568.7, a potential human WISP-1 splice variant, 5' end only, obtained by screening with a probe derived from clone 568.15A.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTSI. Definitions

The term "WISP polypeptide" refers to the family of native- sequence human and mouse WISP proteins and variants described herein whose genes are induced at least by Wnt-1. This term includes WISP-1, WISP-2, and WISP-3.

The terms "WISP-1 polypeptide", "WISP-1 homologue" and grammatical variants thereof, as used herein, encompass native- sequence WISP-1 protein and variants (which are further defined herein). The WISP-1 polypeptide may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods, or by any combination of these and similar techniques.

The terms "WISP-2 polypeptide", "WISP-2 homologue", "PRO261", and "PRO261 polypeptide" and grammatical variants thereof, as used herein, encompass native-sequence WISP-2 protein and variants (which are further defined herein). The WISP-2 polypeptide may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods, or by any combination of these and similar techniques.

The terms "WISP-3 polypeptide", "WISP-3 homologue", and grammatical variants thereof, as used herein, encompass native-sequence WISP-3 protein and variants (which are further defined herein). The WISP-3 polypeptide may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods, or by any combination of these and similar techniques.

A "native-sequence WISP-1 polypeptide" comprises a polypeptide having the same amino acid sequence as a WISP-1 polypeptide derived from nature. Such native-sequence WISP-1 polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native-sequence WISP-1 polypeptide" specifically encompasses naturally occurring truncated or secreted forms of a WISP-1 polypeptide disclosed herein, naturally occurring variant forms (*e.g.*, alternatively spliced forms or splice variants), and naturally occurring allelic variants of a WISP-1 polypeptide. In one embodiment of the invention, the native-sequence WISP-1 polypeptide is a mature or full-length native-sequence human WISP-1 polypeptide comprising amino acids 23 to 267 of Figures 3A and 3B (SEQ ID NO:3) or amino acids 1 to 267 of Figures 3A and 3B (SEQ ID NO:4), respectively, with or without the N-terminal methionine.

In another embodiment of the invention, the native-sequence WISP-1 polypeptide is the full-length or mature native-sequence human WISP-1 polypeptide comprising amino acids 23 to 267 or 1 to 267 of Figures 3A and 3B wherein the valine residue at position 184 or the alanine residue at position 202 has/have been changed to an isoleucine or serine residue, respectively. (SEQ ID NOS:5-8) with or without the N-terminal methionine. In another embodiment of the invention, the native-sequence WISP-1 polypeptide is the full-length or mature native-sequence human WISP-1 polypeptide comprising amino acids 23 to 267 or 1 to 267 of Figures 3A and 3B wherein the valine residue at position 184 and the alanine residue at position 202 has/have been changed to an isoleucine or serine residue, respectively. (SEQ ID NOS:21 and 22, respectively) with or without the N-terminal methionine. In another embodiment of the invention, the native-sequence WISP-1 polypeptide is a mature or full-length native-sequence mouse WISP-1 polypeptide



comprising amino acids 23 to 367 of Figure 1 (SEQ ID NO:11), or amino acids 1 to 367 of Figure 1 (SEQ ID NO:12), respectively, with or without the N-terminal methionine.

In another embodiment of the invention, the native-sequence WISP-1 polypeptide is one which is encoded by a nucleotide sequence comprising one of the human WISP-1 splice or other native-sequence variants, including SEQ ID NOS:23, 24, 25, 26, 27, 28, or 29, with or without an N-terminal methionine.

A "native-sequence WISP-2 polypeptide" or a "native-sequence PRO261 polypeptide" comprises a polypeptide having the same amino acid sequence as a WISP-2 polypeptide derived from nature. Such native-sequence WISP-2 polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native-sequence WISP-2 polypeptide" specifically encompasses naturally occurring truncated or secreted forms of a WISP-2 polypeptide disclosed herein, naturally occurring variant forms (e.g., alternatively spliced forms or splice variants), and naturally occurring allelic variants of a WISP-2 polypeptide. In one embodiment of the invention, the native-sequence WISP-2 polypeptide is a mature or full-length native-sequence human WISP-2 polypeptide comprising amino acids 1-24 up to 250 of Figure 4 (SEQ ID NOS:15, 16, and 56-77), including amino acids 24 to 250 and amino acids 1 to 250 of Figure 4 (SEQ ID NOS:15 and 16, respectively), with or without the N-terminal methionine. In another embodiment of the invention, the native-sequence WISP-2 polypeptide is a mature or full-length native-sequence mouse WISP-2 polypeptide comprising amino acids 1-24 up to 251 of Figure 2 (SEQ ID NOS:19, 20, and 78-99), including amino acids 24 to 251 and amino acids 1 to 251 of Figure 2 (SEQ ID NOS:19 and 20, respectively), with or without the N-terminal methionine.

A "native-sequence WISP-3 polypeptide" comprises a polypeptide having the same amino acid sequence as a WISP-3 polypeptide derived from nature. Such native-sequence WISP-3 polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native-sequence WISP-3 polypeptide" specifically encompasses naturally occurring truncated or other forms of a WISP-3 polypeptide disclosed herein, naturally occurring variant forms (e.g., alternatively spliced forms or splice variants), and naturally occurring allelic variants of a WISP-3 polypeptide. In one embodiment of the invention, the native-sequence WISP-3 polypeptide is a mature or full-length native-sequence human WISP-3 polypeptide comprising amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32) or amino acids 1 to 372 of Figures 6A and 6B (SEQ ID NO:33), respectively, with or without the N-terminal methionine. In another embodiment of the invention, the native-sequence WISP-3 polypeptide is a mature or full-length native-sequence human WISP-3 polypeptide comprising amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36) or amino acids 1 to 355 of Figures 7A and 7B (SEQ ID NO:37), respectively, with or without the N-terminal methionine.

The term "WISP-1 variant" means an active WISP-1 polypeptide as defined below having at least about 80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95% amino acid sequence identity with human mature WISP-1 having the deduced amino acid sequence shown in Figs. 3A and 3B (SEQ ID NO:3), and/or with human full-length WISP-1 having the deduced amino acid sequence shown in Figs. 3A and 3B (SEQ ID NO:4), and/or with mouse mature WISP-1 having the deduced amino acid sequence shown in Fig. 1 (SEQ ID NO:11), and/or with mouse full-length WISP-2 having the deduced amino acid sequence shown in Fig. 1 (SEQ ID NO:12). Such variants include, for

instance, WISP-1 polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length or mature sequences of Figures 3A-3B and 1 (SEQ ID NOS:4, 3, 12, and 11, respectively), including variants from other species, but excludes a native-sequence WISP-1 polypeptide.

The term "WISP-2 variant" or "PRO261 variant" means an active WISP-2 polypeptide as defined below having at least about 80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95% amino acid sequence identity with human mature WISP-2 having the putative deduced amino acid sequence shown in Fig. 4 (SEQ ID NO:15), and/or with human full-length WISP-2 having the deduced amino acid sequence shown in Fig. 4 (SEQ ID NO:16), and/or with mouse mature WISP-2 having the putative deduced amino acid sequence shown in Fig. 2 (SEQ ID NO:19), and/or with mouse full-length WISP-2 having the deduced amino acid sequence shown in Fig. 2 (SEQ ID NO:20). Such variants include, for instance, WISP-2 polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length and putative mature sequences of Figures 4 and 2 (SEQ ID NOS:16, 15, 20, and 19, respectively), including variants from other species, but excludes a native-sequence WISP-2 polypeptide.

The term "WISP-3 variant" means an active WISP-3 polypeptide as defined below having at least about 80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95% amino acid sequence identity with human mature WISP-3 having the deduced amino acid sequence shown in Figs. 6A and 6B (SEQ ID NO:32), and/or with human full-length WISP-3 having the deduced amino acid sequence shown in Figs. 6A and 6B (SEQ ID NO:33), and/or with human mature WISP-3 having the deduced amino acid sequence shown in Figs. 7A and 7B (SEQ ID NO:36), or with human full-length WISP-3 having the deduced amino acid sequence shown in Figs. 7A and 7B (SEQ ID NO:37). Such variants include, for instance, WISP-3 polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length or mature sequences of Figures 6A-6B and 7A-7B (SEQ ID NOS:32, 33, 36, and 37, respectively), including variants from other species, but excludes a native-sequence WISP-3 polypeptide.

"Percent (%) amino acid sequence identity" with respect to the WISP sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a WISP polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN, or Megalign (DNASTAR<sup>TM</sup>) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

"Percent (%) nucleic acid sequence identity" with respect to the coding region of the WISP sequences identified herein, including UNQ228 (DNA34387-seq min) sequence, and the coding region therein, is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the coding region of the WISP sequence of interest, e.g., in the UNQ228 (DNA34387-seq min) sequence (SEQ ID NO:38) or coding region therein (SEQ ID NO:16), after aligning the sequences and

introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS; or (4) employ a buffer of 10% dextran sulfate, 2 x SSC (sodium chloride/sodium citrate), and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" are described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989), and include the use of a washing solution and hybridization conditions (*e.g.*, temperature, ionic strength, and percent SDS) less stringent than described above. An example of moderately stringent conditions is a condition such as overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, *etc.*, as necessary to accommodate factors such as probe length and the like.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the WISP natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" nucleic acid encoding a WISP polypeptide or "isolated" DNA33473 or "isolated" PRO261 polypeptide-encoding nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the respective nucleic acid. Isolated DNA33473 or an isolated WISP-encoding nucleic acid

molecule is other than in the form or setting in which it is found in nature. An isolated WISP-encoding or DNA33-473 nucleic acid molecule therefore is distinguished from the WISP-encoding or DNA33-473 nucleic acid molecule, respectively, as it exists in natural cells. However, an isolated WISP-encoding or DNA33-473 nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express WISP-encoding DNA or DNA33-473, respectively, where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers single anti-WISP polypeptide, such as anti-PRO261, monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), and anti-WISP polypeptide, such as anti-PRO261, and antibody compositions with polypeptidic specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

"Active" or "activity" or "WISP biological activity", for purposes herein, describes form(s) of a WISP polypeptide, such as PRO261, including its variants, or its antagonists, which retain the biologic and/or immunologic activities of a native or naturally occurring (native-sequence) WISP polypeptide, such as PRO261, or its antagonist. Preferred "activities" for a WISP polypeptide or its antagonist include the ability to inhibit proliferation of tumor cells or to stimulate proliferation of normal cells and to treat arteriosclerosis, including atherosclerosis, as well as to induce wound repair and hematopoiesis, prevent desmoplasia, prevent fibrotic lesions associated with skin disorders such as scleroderma, keloid, eosinophilic fasciitis, nodular fasciitis, and Dupuytren's contracture, to treat bone-related diseases such as osteoporosis, to regulate anabolism including promotion of growth, to treat immune disorders, to treat Wilms' tumor and kidney-related disorders, to treat testis-related disorders, to treat lung-related disorders, and to treat cardiac disorders.

An "antagonist" of a WISP polypeptide is a molecule that inhibits an activity of a WISP polypeptide. Preferred antagonists are those which interfere with or block an undesirable biological activity of a WISP polypeptide, such as where a WISP polypeptide might act to stimulate cancer cells and the antagonist would serve to inhibit the growth of those cells. In some cases, such as with WISP-1, WISP-2, and WISP-3, the

antagonist may be useful to inhibit the binding of a WISP polypeptide to an IGF. Such molecules include antibodies and small molecules that have such inhibitory capability, as well as WISP polypeptide variants of, and receptors for, WISP polypeptide (if available) or portions thereof that bind to WISP. For example, antagonists can be derived from receptors of WISP-1, WISP-2, and WISP-3 using the predicted family of receptors for WISPs-1, -2, and -3 (the CTGF receptors). Thus, the receptor can be expression cloned from the family; then a soluble form of the receptor is made by identifying the extracellular domain and excising the transmembrane domain therefrom. The soluble form of the receptor can then be used as an antagonist, or the receptor can be used to screen for small molecules that would antagonize WISP polypeptide activity.

Alternatively, using the murine sequences shown in Figures 1 and 2 (SEQ ID NOS: 11, 12, 19, and 20, respectively) or the human sequences shown in Figures 3A-3B, 4, (SEQ ID NOS: 3, 4, 15, and 16, respectively), 6A-6B, and 7A-7B, variants of native WISP-1, WISP-2, or WISP-3, are made that act as antagonists. Using knowledge from the CTGF receptor family, the receptor binding sites of WISP-1, WISP-2, and WISP-3 polypeptides can be determined by binding studies and one of them eliminated by standard techniques (deletion or radical substitution) so that the molecule acts as an antagonist.

Antagonist activity can be determined by several means, including standard assays for induction of cell death such as that described herein, *e.g.*,  $^3\text{H}$ -thymidine proliferation assays, or other mitogenic assays, such as an assay measuring the capability of the candidate antagonist of inducing EGF-potentiased anchorage independent growth of target cell lines (Volckaert *et al.*, Gene, **15**:215-223 (1981)) and/or growth inhibition of neoplastic cell lines. Roberts *et al.*, Proc. Natl. Acad. Sci. USA, **82**:119-123 (1985). Anchorage-independent growth refers to the ability of WISP polypeptide-treated or TGF- $\beta$ -treated and EGF-treated non-neoplastic target cells to form colonies in soft agar, a characteristic ascribed to transformation of the cells. In this assay, the candidate is incubated together with an equimolar amount of a WISP polypeptide otherwise detectable in the EGF-potentiased anchorage-independent target cell growth assay, and the culture observed for failure to induce anchorage-independent growth. In addition, an antagonist may be an IGF such as IGF-I or a peptide mimic of IGF-I or a receptor to IGF or a receptor to an IGFBP.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder or condition as well as those in which the disorder or condition is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic, and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, sheep, pigs, cows, *etc.* Preferably, the mammal is human.

A "disorder" or "WISP-related disorder" is any condition that would benefit from treatment with the WISP polypeptides or WISP antagonists herein. This includes chronic and acute disorders, as well as those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal, and blastocoelic disorders; hematopoiesis-related disorders; tissue-growth disorders; skin disorders; desmoplasia, fibrotic lesions; kidney disorders; bone-related disorders; trauma such as burns, incisions, and other wounds; catabolic states; testicular-related disorders; and inflammatory, angiogenic, and immunologic disorders.

including arteriosclerosis. A "Wnt-related disorder" is one caused at least by the upregulation of the Wnt gene pathway, including Wnt-1 and Wnt-4, but preferably Wnt-1, and may include cancer.

The terms "cancer", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma including adenocarcinoma, lymphoma, blastoma, melanoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's and non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer such as hepatic carcinoma and hepatoma, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer such as renal cell carcinoma and Wilms' tumors, basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, and various types of head and neck cancer. The preferred cancers for treatment herein are breast, colon, lung, and melanoma.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g.,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{90}\text{Y}$ , and  $^{186}\text{Re}$ ), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiopeta, Busulfan, Cytosin, Taxol, Taxotere, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincristine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors, such as tamoxifen and onapristone.

A "growth-inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, such as a Wnt-overexpressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth-inhibitory agent is one which significantly reduces the percentage of malignant cells in S phase. Examples of growth-inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The 4D5 antibody (and functional equivalents thereof) can also be employed for this purpose if the cancer involves ErbB2-overexpressing cancer cells. See, e.g., WO 92/22653.

"Northern analysis" or "Northern blot" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment.

The probe is labeled with a radioisotope such as  $^{32}\text{P}$ , or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

5 The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The  
10 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage, or plasmid sequences, *etc.* See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol., 51: 263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase  
15 reaction method for amplifying a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

## II. Compositions and Methods of the Invention

### A. Full-length WISP Polypeptide

The present invention provides newly-identified and isolated nucleotide sequences encoding a  
20 polypeptide referred to in the present application as a WISP polypeptide, including a WISP-1, WISP-2, or WISP-3 polypeptide. In particular, cDNAs have been identified and isolated encoding novel murine and human WISP-1 and WISP-2, and human WISP-3 splice variants as disclosed in further detail in the Examples below.

Using BLAST and FastA sequence alignment computer programs, it was found that the coding  
25 sequences of mouse and human WISP-1 and -2, as well as the two coding sequences of human WISP-3 disclosed herein, show significant homology to DNA sequences disclosed in the GenBank database, including those published by Adams *et al.*, Nature, 377: 3-174 (1995).

Further, using BLAST and FastA sequence alignment computer programs, it was found that various  
30 portions of the coding sequences of mouse and human WISP-1 and WISP-2 show significant homology to CTGF, cef-10, Cyr61, and/or Nov protein. In this regard, mouse WISP-1 is 47% homologous to mouse CTGF and 46% homologous to human CTGF, mouse WISP-2 is 46% homologous to chick cef-10 protein precursor and 42% homologous to human Cyr61 protein, human WISP-1 is 47% homologous to mouse CTGF and 48% homologous to human CTGF, and human WISP-2 is 48% homologous to mouse CTGF, 49% homologous to human CTGF precursor, 46% homologous to mouse Nov protein homolog precursor, 49%  
35 homologous to human CTGF, and 48% homologous to mouse CTGF precursor. Further, apparently the amino acid sequences of mouse WISP-1 and mouse ELM1 (Hashimoto *et al.*, *supra*) are identical, and the amino acid sequences of human WISP-1 and mouse ELM1 are 84% identical.

Since these factors have also been correlated with IGFBPs, it is presently believed that the WISP-1 and WISP-2 polypeptides disclosed in the present application are newly identified members of the CTGF or

IGFBP family and possess activity relating to development of normal, injured, and cancerous cells and tissue. More specifically, WISP-1 and WISP-2 may be involved in breast cancer, lung cancer, melanoma, and colon cancer, as well as in wound repair. Further, they may be involved in atherosclerosis.

Further, using BLAST and FastA sequence alignment computer programs, it was found that various portions of the coding sequences of the two splice variants of human WISP-3 show significant homology to mouse ELM1 and CTGF proteins. In this regard, both splice variants of WISP-3 are 45% homologous to mouse ELM1 and 42% homologous to mouse and human CTGF and its precursor, with the longer variant of Fig. 6 being 43% homologous to *Xenopus* CTGF and the shorter variant of Fig. 7 being 42% homologous to *Xenopus* CTGF.

#### B. WISP Polypeptide Variants

In addition to the full-length native-sequence WISP polypeptides described herein, it is contemplated that variants of these sequences can be prepared. WISP variants can be prepared by introducing appropriate nucleotide changes into the WISP-encoding DNA, or by synthesis of the desired variant WISP polypeptides. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the WISP polypeptide, such as changing the number or position of glycosylation sites or altering the membrane-anchoring characteristics, if the native WISP polypeptide is membrane bound.

Variations in the native full-length WISP sequences, or in various domains of the WISP polypeptides described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion, or insertion of one or more codons encoding the WISP polypeptide that results in a change in the amino acid sequence as compared with the native-sequence WISP polypeptide. Optionally the variation is by substitution of at least one amino acid with any other amino acid in any portion of the WISP polypeptide. Guidance in determining which amino acid residue may be inserted, substituted, or deleted without adversely affecting the desired activity may be found by comparing the sequence of the WISP polypeptide with that of homologous known CTGF protein molecules, in the case of WISP-1, WISP-2, and WISP-3, and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to about 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions, or substitutions of amino acids in the sequence and testing the resulting variants for activity in *in vitro* assays for gene upregulation or downregulation and in transgenic or knockout animals.

The variations can be made on the cloned DNA to produce the WISP DNA or WISP polypeptide variant DNA using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis (Carter *et al.*, Nucl. Acids Res., **13**:4331 (1986); Zoller *et al.*, Nucl. Acids Res., **10**:6487 (1987)), cassette mutagenesis (Wells *et al.*, Gene, **34**:315 (1985)), alanine scanning, PCR mutagenesis, restriction selection mutagenesis (Wells *et al.*, Philos. Trans. R. Soc. London SerA, **317**:415 (1986)), or other known techniques.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids.



Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions. T.E. Creighton, *Proteins: Structure and Molecular Properties* (W.H. Freeman & Co., San Francisco, 1983); Chothia, *J. Mol. Biol.*, **150**:1 (1976). If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

Further deletional variants of the full-length WISP polypeptide include variants from which the N-terminal signal peptide, if any (such as, for example, those putatively identified as amino acids 1 to 22 for WISP-1, 1 to 23 for WISP-2, 1-33 for the WISP-3 of Fig. 6 and 1-15 for the WISP-3 of Fig. 7), and/or the initiating methionine has been deleted.

#### C. Modifications of the WISP Polypeptide

Covalent modifications of the WISP polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a WISP polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues. Derivatization with bifunctional agents is useful, for instance, for crosslinking a WISP polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-WISP antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidylesters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-((p-azidophenyl)-dithio)propioimide.

Other modifications include deamidation of glutamyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively; hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains (Creighton, *supra*, pp. 79-86), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group. Another type of covalent modification of the WISP polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in the native sequence (either by deleting the underlying glycosylation site or by removing the glycosylation moieties by chemical and/or enzymatic means) and/or adding one or more glycosylation sites that are not present in the native sequence. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportion of the various sugar residues present.

Addition of glycosylation sites to the WISP polypeptide herein may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence (for O-linked glycosylation sites). The amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA

encoding the WISP polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above.

Another means of increasing the number of carbohydrate moieties on the WISP polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the WISP polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, Arch. Biochem. Biophys., 259:52 (1987) and by Edge *et al.*, Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, Meth. Enzymol., 138:350 (1987).

Another type of covalent modification comprises linking the WISP polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth, e.g., in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The WISP polypeptide of the present invention may also be modified in a way to form a chimeric molecule comprising a WISP polypeptide, or a fragment thereof, fused to a heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of the WISP polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of a native or variant WISP molecule. The presence of such epitope-tagged forms can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the WISP polypeptides to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimeric molecule may comprise a fusion of the WISP polypeptides, or fragments thereof, with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an Ig, such as an IgG molecule.

Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-His) or poly-histidine-glycine (poly-His-Gly) tags; the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, Mol. Cell. Biol., 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7, and 9E10 antibodies thereto (Evan *et al.*, Molecular and Cellular Biology, 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody. Paborsky *et al.*, Protein Engineering, 3(6):547-553 (1990). Other tag polypeptides include the Flag-peptide (Hopp *et al.*, BioTechnology, 6:1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.*, Science, 255:192-194 (1992)); an  $\alpha$ -tubulin epitope peptide (Skinner *et al.*, J. Biol. Chem., 266:15163-15166 (1991)); and the T7 gene 10 protein peptide tag. Lutz-Freyermuth *et al.*, Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990).

#### D. Preparation of WISP Polypeptide

The description below relates primarily to production of WISP polypeptides by culturing cells transformed or transfected with a vector containing at least DNA encoding the mature or full-length sequences

of human or mouse WISP-1 (SEQ ID NOS:3, 4, 11, or 12, respectively), or containing at least DNA encoding the mature or full-length sequences of human or mouse WISP-2 (SEQ ID NOS:15, 16, 19, or 20, respectively), or containing at least DNA encoding the mature or full-length sequences of human WISP-3 of Fig. 6 (SEQ ID NOS:32 or 33, respectively), or containing at least DNA encoding the mature or full-length sequences of human WISP-3 of Fig. 7 (SEQ ID NOS:36 or 37, respectively).

It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare WISP polypeptides. For instance, the WISP polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques. See, e.g., Stewart *et al.*, Solid-Phase Peptide Synthesis (W.H. Freeman Co.: San Francisco, CA, 1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems peptide synthesizer (Foster City, CA) in accordance with manufacturer's instructions. Various portions of WISP polypeptides may be chemically synthesized separately and combined using chemical or enzymatic methods to produce a full-length WISP polypeptide.

#### 1. Isolation of DNA Encoding WISP Polypeptide

DNA encoding a WISP polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the mRNA for WISP polypeptide and to express it at a detectable level. Accordingly, DNA encoding human WISP polypeptide can be conveniently obtained from a cDNA library prepared from human tissue, such as a human fetal liver library or as otherwise described in the Examples. The gene encoding WISP polypeptide may also be obtained from a genomic library or by oligonucleotide synthesis.

A still alternative method of cloning WISP polypeptide is suppressive subtractive hybridization, which is a method for generating differentially regulated or tissue-specific cDNA probes and libraries. This is described, for example, in Diatchenko *et al.*, Proc. Natl. Acad. Sci. USA, 93: 6025-6030 (1996). The procedure is based primarily on a technique called suppression PCR and combines normalization and subtraction in a single procedure. The normalization step equalizes the abundance of cDNAs within the target population and the subtraction step excludes the common sequences between the target and driver populations.

Libraries can be screened with probes (such as antibodies to a WISP polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *supra*. An alternative means to isolate the gene encoding WISP polypeptide is to use PCR methodology. Sambrook *et al.*, *supra*; Dieffenbach *et al.*, PCR Primer: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1995).

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation, or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as ALIGN, DNASTar, and INHERIT which employ various algorithms to measure homology.

Nucleic acid having polypeptide-coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequences disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

## 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for WISP polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH, and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example,  $\text{CaPO}_4$  and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene or polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, Methods in Enzymology, 185:527-537 (1990) and Mansour *et al.*, Nature, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31.446); *E. coli* X1776 (ATCC 31.537); *E. coli* strain W3110 (ATCC 27.325); and K5 772 (ATCC 53.635). These examples

are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT karF*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rhs7 ilvG karF*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for vectors containing nucleic acid encoding WISP polypeptide. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, Bio/Technology, 9: 968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, J. Bacteriol., 737 (1983)), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906; Van den Berg *et al.*, Bio/Technology, 8: 135 (1990)), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 (1988)); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, Proc. Natl. Acad. Sci. USA, 76: 5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, Biochem. Biophys. Res. Commun., 112: 284-289 (1983); Tilburn *et al.*, Gene, 26: 205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81: 1470-1474 (1984)) and *A. niger* Kelly and Hynes, EMBO J., 4: 475-479 (1985). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylotrophs, 269 (1982).

Suitable host cells for the expression of glycosylated WISP are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture (Graham *et al.*, J. Gen. Virol., 36: 59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23: 243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor

(MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding the desired WISP polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The desired WISP polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence, if the WISP polypeptide is conducive to being secreted, or other polypeptide having a specific cleavage site at the N-terminus of the mature or full-length protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the DNA encoding the WISP polypeptide that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence such as, for example, the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, and including signals from WISP polypeptides.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV, or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the nucleic acid encoding WISP polypeptide, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7.

Stinchcomb *et al.*, Nature, 282:39 (1979); Kingsman *et al.*, Gene, 7:141 (1979); Tschemper *et al.*, Gene, 10:157 (1980). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85:12 (1977).

Expression and cloning vectors usually contain a promoter operably linked to the nucleic acid sequence encoding WISP polypeptide to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems (Chang *et al.*, Nature, 275:615 (1978); Goeddel *et al.*, Nature, 281:544 (1979)); alkaline phosphatase, a tryptophan (*trp*) promoter system (Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776), and hybrid promoters such as the *tac* promoter. deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80:21-25 (1983). Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the WISP polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 (1980)) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

WISP transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, and Simian Virus 40 (SV40); from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter; and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding a WISP polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the sequence coding for a WISP polypeptide, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding WISP polypeptide.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of WISP polypeptides in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, 293:620-625 (1981); Mantei *et al.*, Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native-sequence WISP polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to DNA encoding WISP polypeptide and encoding a specific antibody epitope.

#### 5. Purification of Polypeptide

Forms of WISP polypeptide may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g., Triton-X 100) or by enzymatic cleavage. Cells employed in expression of WISP polypeptides can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify WISP polypeptide from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, SEPHADEX<sup>TM</sup> G-75; protein A SEPHAROSE<sup>TM</sup> columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the WISP polypeptide. Various methods of protein purification may be employed, and such methods are known in the art and described, for example,



in Deutscher, Methods in Enzymology, 182 (1990); and Scopes, Protein Purification: Principles and Practice (Springer-Verlag: New York, 1982).

In one specific example of purification, either a poly-His tag or the Fc portion of human IgG is added to the C-terminal coding region of the cDNA for WISP-1, WISP-2, or WISP-3 before expression. The conditioned media from the transfected cells are harvested by centrifugation to remove the cells and filtered. For the poly-His-tagged constructs, the protein may be purified using a Ni-NTA column. After loading, the column may be washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein may then be desalted into a storage buffer if desired.

Immunoadhesin (Fc-containing) constructs of the WISP-1, WISP-2, and WISP-3 proteins may be purified from the conditioned media by pumping them onto a 5-ml Protein A column which had been equilibrated in a phosphate buffer. After loading, the column may be washed extensively with equilibration buffer before elution with citric acid. The eluted protein may be immediately neutralized by collecting 1-ml fractions into tubes containing TRIS buffer. The highly purified protein may be subsequently desalted into storage buffer as described above for the poly-His-tagged proteins. The homogeneity of the protein may be assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

The purification step(s) selected will depend, for example, on the nature of the production process used and the particular WISP polypeptide produced.

#### E. Uses for WISP Polypeptide and Its Nucleic Acid

Nucleotide sequences (or their complement) encoding WISP polypeptides have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping, and in the generation of anti-sense RNA and DNA. Nucleic acid encoding WISP polypeptide will also be useful for the preparation of WISP polypeptides by the recombinant techniques described herein.

The full-length nucleotide sequences for mouse or human WISP-1 or WISP-2 (SEQ ID NOS:9, 1, 17, and 13, respectively), or portions thereof, or the full-length nucleotide sequences for human WISP-3 of Fig. 6 (SEQ ID NO:30) or for WISP-3 of Fig. 7 (SEQ ID NO:34) may be used as hybridization probes for a cDNA library to isolate or detect the full-length gene encoding the WISP polypeptide of interest or to isolate or detect still other genes (for instance, those encoding naturally occurring variants of WISP polypeptide, other WISP polypeptide family members, or WISP polypeptides from other species) which have a desired sequence identity to the WISP polypeptide sequences disclosed in Figures 1, 2, 3A and 3B, 4, 6A and 6B, and 7A and 7B (SEQ ID NOS:3, 4, 11, 12, 15, 16, 19, 20, 32, 33, 36, or 37). For example, such procedures as *in situ* hybridization, Northern and Southern blotting, and PCR analysis may be used to determine whether DNA and/or RNA encoding a different WISP is present in the cell type(s) being evaluated. Optionally, the length of the probes will be about 20 to about 50 bases. For example, the hybridization probes may be derived from the UNQ228 (DNA33473-seq min) nucleotide sequence (SEQ ID NO:38) or the full-length human WISP-2 nucleotide sequence (SEQ ID NO:13) as shown in Figure 4 or from genomic sequences including promoters, enhancer elements, and introns of DNA encoding native-sequence WISP polypeptide.

By way of example, a screening method will comprise isolating the coding region of the WISP gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of any of the genes encoding WISP polypeptides of the present invention can be used to screen libraries of human cDNA, genomic DNA, or mRNA to determine to which members of such libraries the probe hybridizes. Hybridization techniques are described in further detail in the Examples below.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related WISP sequences.

Nucleotide sequences encoding a WISP polypeptide can also be used to construct hybridization probes for mapping the gene which encodes that WISP polypeptide and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries. If the amplification of a given gene is functionally relevant, then that gene should be amplified more than neighboring genomic regions which are not important for tumor survival. To test this, the gene can be mapped to a particular chromosome, e.g., by radiation-hybrid analysis. The amplification level is then determined at the location identified, and at neighboring genomic region. Selective or preferential amplification at the genomic region to which the gene has been mapped is consistent with the possibility that the gene amplification observed promotes tumor growth or survival. Chromosome mapping includes both framework and epicenter mapping. For further details see e.g., Stewart *et al.*, Genome Research 7, 422-433 (1997).

Nucleic acid encoding a WISP polypeptide may be used as a diagnostic to determine the extent and rate of the expression of the DNA encoding the WISP polypeptide in the cells of a patient. To accomplish such an assay, a sample of a patient's cells is treated, via *in situ* hybridization, or by other suitable means, and analyzed to determine whether the sample contains mRNA molecules capable of hybridizing with the nucleic acid molecule.

Nucleic acids which encode WISP polypeptides or any of their modified forms can also be used to generate either transgenic animals or "knock-out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding a WISP polypeptide can be used to clone genomic DNA encoding the WISP polypeptide in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding the WISP polypeptide.

Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009 and WO 97/38086. Typically, particular cells would be targeted for WISP transgene incorporation with tissue-specific

enhancers. Transgenic animals that include a copy of a transgene encoding the WISP polypeptide introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding the WISP polypeptide. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of WISP polypeptides can be used to construct a WISP polypeptide "knock-out" animal which has a defective or altered gene encoding a WISP polypeptide as a result of homologous recombination between the endogenous gene encoding the WISP polypeptide and altered genomic DNA encoding the WISP polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding the WISP polypeptide can be used to clone genomic DNA encoding the WISP polypeptide in accordance with established techniques. A portion of the genomic DNA encoding the WISP polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector. See e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected. See e.g., Li et al., Cell, 69:915 (1992). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras. See e.g., Bradley, in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock-out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized, for instance, by their ability to defend against certain pathological conditions and by their development of pathological conditions due to absence of the WISP polypeptide.

In particular, assays in which CTGF, IGFBPs, and other members of the CTGF superfamily and other growth factors are usually used are preferably performed with the WISP-1 and WISP-2 polypeptides. For example, an assay to determine whether TGF- $\beta$  induces the WISP polypeptide, indicating a role in cancer, may be performed as known in the art, as well as assays involving induction of cell death and  $^3\text{H}$ -thymidine proliferation assays. Mitogenic and tissue growth assays are also performed with the WISP polypeptide as set forth above. The results are applied accordingly.

The WISP polypeptides of the present invention may also be used to induce the formation of anti-WISP polypeptide antibodies, which are identified by routine screening as detailed below.

In addition to their uses above, the WISP-1, WISP-2, and WISP-3 polypeptides of the present invention are useful as the basis for assays of IGF activity. Importantly, since such an assay measures a physiologically significant binding event, i.e., that of an IGF to its IGFBP, triggering a detectable change (such as phosphorylation, cleavage, chemical modification, etc.), it is likely to be both more sensitive and

more accurate than immunoassays, which detect the physiologically non-significant binding of an IGF to anti-WISP polypeptide antibody. Although more sensitive and accurate than antibodies, the WISP-1, WISP-2, and WISP-3 molecules of the invention can be used to assay IGF (such as IGF-I or IGF-II) levels in a sample in the same ways in which antibodies are used.

5 For diagnostic purposes, the WISP-1, WISP-2, or WISP-3 polypeptide can be used in accordance with immunoassay technology. Examples of immunoassays are provided by Wide at pages 199-206 of Radioimmune Assay Method, Kirkham and Huner, ed., E & S. Livingstone, Edinburgh, 1970.

Thus, in one embodiment, WISP-1, WISP-2, and WISP-3 polypeptides can be detectably labeled and incubated with a test sample containing IGF molecules (such as biological fluids, e.g., serum, sputum, urine, etc.), and the amount of WISP-1, WISP-2, or WISP-3 molecule bound to the sample ascertained.

10 Immobilization of reagents is required for certain assay methods. Immobilization entails separating the WISP-1, WISP-2, or WISP-3 polypeptide from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the WISP-1, WISP-2, or WISP-3 polypeptide before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich *et al.*, U.S. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the molecule

15 afterward, e.g., by immunoprecipitation. The foregoing are merely exemplary diagnostic assays for IGF. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof.

WISP-1, WISP-2, and WISP-3 polypeptides are also useful in radioimmunoassays to measure IGFs such as IGF-I or IGF-II. Such a radioimmunoassay would be conducted as described in the literature using the naturally purified or recombinant WISP-1, WISP-2, or WISP-3 as the WISP element.

20 In addition, WISP polypeptides are useful for screening for compounds that bind to them as defined above. Preferably, these compounds are small molecules such as organic or peptide molecules that exhibit one or more of the desired activities. Screening assays of this kind are conventional in the art, and any such screening procedure may be employed, whereby the test sample is contacted with the WISP polypeptide herein and the extent of binding and biological activity of the bound molecule are determined.

25 More specifically, this invention encompasses methods of screening compounds to identify those that mimic the WISP polypeptide (agonists) or prevent the effect of the WISP polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the WISP polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

30 The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

All assays for antagonists are common in that they call for contacting the drug candidate with a WISP polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the WISP polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the WISP polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the WISP polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular WISP polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, *e.g.*, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers (Fields and Song, Nature (London), 340: 245-246 (1989); Chien *et al.*, Proc. Natl. Acad. Sci. USA, 88: 9578-9582 (1991)) as disclosed by Chevray and Nathans, Proc. Natl. Acad. Sci. USA, 89: 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, the other one functioning as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER<sup>TM</sup>) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a WISP polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the

mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

If the WISP polypeptide has the ability to stimulate the proliferation of endothelial cells in the presence of the co-mitogen ConA, then one example of a screening method takes advantage of this ability. Specifically, in the proliferation assay, human umbilical vein endothelial cells are obtained and cultured in 96-well flat-bottomed culture plates (Costar, Cambridge, MA) and supplemented with a reaction mixture appropriate for facilitating proliferation of the cells, the mixture containing Con-A (Calbiochem, La Jolla, CA). Con-A and the compound to be screened are added and after incubation at 37°C, cultures are pulsed with  $^3\text{H}$ -thymidine and harvested onto glass fiber filters (pH: Cambridge Technology, Watertown, MA). Mean  $^3\text{H}$ -thymidine incorporation (cpm) of triplicate cultures is determined using a liquid scintillation counter (Beckman Instruments, Irvine, CA). Significant  $^3\text{H}$ -thymidine incorporation indicates stimulation of endothelial cell proliferation.

To assay for antagonists, the assay described above is performed; however, in this assay the WISP polypeptide is added along with the compound to be screened and the ability of the compound to inhibit  $^3\text{H}$ -thymidine incorporation in the presence of the WISP polypeptide indicates that the compound is an antagonist to the WISP polypeptide. Alternatively, antagonists may be detected by combining the WISP polypeptide and a potential antagonist with membrane-bound WISP polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The WISP polypeptide can be labeled, such as by radioactivity, such that the number of WISP polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan *et al.*, Current Protocols in Immun., 1(2): Chapter 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the WISP polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the WISP polypeptide. Transfected cells that are grown on glass slides are exposed to labeled WISP polypeptide. The WISP polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled WISP polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled WISP polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be measured.

The compositions useful in the treatment of WISP-related disorders include, without limitation, antibodies, small organic and inorganic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple-helix molecules, etc., that inhibit the expression and/or activity of the target gene product.

More specific examples of potential antagonists include an oligonucleotide that binds to the WISP polypeptide, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the WISP polypeptide that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the WISP polypeptide.

Another potential WISP polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature WISP polypeptides herein, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, Nucl. Acids Res., 6: 3073 (1979); Cooney *et al.*, Science, 241: 456 (1988); Dervan *et al.*, Science, 251: 1360 (1991)), thereby preventing transcription and the production of the WISP polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule to the WISP polypeptide (antisense - Okano, Neurochem., 56: 560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the WISP polypeptide. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the WISP polypeptide, thereby blocking the normal biological activity of the WISP polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g., Rossi, Current Biology, 4: 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, *e.g.*, PCT publication

5 No. WO 97/33551, *supra*.

These small molecules can be identified by any one or more of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

WISP-1, WISP-2, and WISP-3 polypeptides are additionally useful in affinity purification of an IGF that binds to WISP-1, WISP-2, or WISP-3 (such as, for example, IGF-I) and in purifying antibodies thereto. The WISP-1, WISP-2, or WISP-3 is typically coupled to an immobilized resin such as Affi-Gel 10™ (Bio-  
10 Rad, Richmond, CA) or other such resins (support matrices) by means well known in the art. The resin is equilibrated in a buffer (such as one containing 150 mM NaCl, 20 mM HEPES, pH 7.4 supplemented to contain 20% glycerol and 0.5% NP-40) and the preparation to be purified is placed in contact with the resin, whereby the molecules are selectively adsorbed to the WISP-1, WISP-2, or WISP-3 on the resin.

15 The resin is then sequentially washed with suitable buffers to remove non-adsorbed material, including unwanted contaminants, from the mixture to be purified, using, *e.g.*, 150 mM NaCl, 20 mM HEPES, pH 7.4, containing 0.5% NP-40; 150 mM NaCl, 20 mM HEPES, pH 7.4 containing 0.5 M NaCl and 0.1% NP-40; 150 mM NaCl, 20 mM HEPES, pH 7.4 containing 0.1% deoxycholate; 150 mM NaCl, 20 mM HEPES, pH 7.4 containing 0.1% NP-40; and a solution of 0.1% NP-40, 20% glycerol and 50 mM glycine,  
20 pH 3. The resin is then treated so as to elute the IGF using a buffer that will break the bond between the IGF and WISP-1, WISP-2, or WISP-3 (using, *e.g.*, 50 mM glycine, pH 3, 0.1% NP-40, 20% glycerol, and 100 mM NaCl).

It is contemplated that the WISP polypeptides of the present invention may be used to treat various conditions, including those characterized by overexpression and/or activation of at least the Wnt pathway. Further, since the WISP-1, WISP-2, and WISP-3 molecules respond to hormone-expressed breast cancer in  
25 mice and are abnormally expressed in human cancer, and are over-amplified in various colon cancer cell lines, they are useful in diagnosing cancer, for example, as a marker for increased susceptibility to cancer or for having cancer. Exemplary conditions or disorders to be treated with the WISP polypeptides include benign or malignant tumors (*e.g.*, renal, liver, kidney, bladder, testicular, breast, gastric, ovarian, colorectal, prostate, pancreatic, lung, esophageal, vulval, thyroid, hepatic carcinomas; sarcomas; glioblastomas; and various head  
30 and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic, and other glandular, macrophagal, epithelial, stromal, and blastocoelic disorders; cardiac disorders; renal disorders; catabolic disorders; bone-related disorders such as osteoporosis; and inflammatory, angiogenic, and immunologic disorders, such as arteriosclerosis; as well as connective tissue disorders,  
35 including wound healing.

The WISP polypeptides of the invention are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular,



intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the polypeptide is preferred.

Therapeutic formulations of the WISP polypeptide are prepared for storage by mixing the polypeptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose, or sorbitol; salt-forming counterions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

Other therapeutic regimens may be combined with the administration of the WISP polypeptides of the instant invention. For example, the patient to be treated with the polypeptides disclosed herein may also receive radiation therapy if the disorder is cancer. Alternatively, or in addition, a chemotherapeutic agent may be administered to the patient with cancer. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, Ed., M.C. Perry (Williams & Wilkins: Baltimore, MD, 1992). The chemotherapeutic agent may precede or follow administration of the polypeptide or may be given simultaneously therewith. The polypeptide may be combined with an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

It may be desirable also to co-administer with the WISP polypeptide (or anti-WISP polypeptide) antibodies against other tumor-associated antigens, such as antibodies which bind to HER-2, EGFR, ErbB2, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more different anti-cancer antibodies, such as anti-ErbB2 antibodies, may be co-administered to the patient with the WISP polypeptide (or anti-WISP polypeptide antibody). Sometimes, it may be beneficial also to administer one or more cytokines to the patient.

In a preferred embodiment, the WISP polypeptide is co-administered with a growth-inhibitory agent to the cancer patient. For example, the growth-inhibitory agent may be administered first, followed by the WISP polypeptide. However, simultaneous administration or administration of the WISP polypeptide first is also contemplated. Suitable dosages for the growth-inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth-inhibitory agent and polypeptide. The

antibodies, cytotoxic agents, cytokines, or growth-inhibitory agents are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. (1980), *supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated polypeptides remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

For the prevention or treatment of disease or disorder, the appropriate dosage of WISP polypeptide will depend on the type of disorder to be treated, as defined above, the severity and course of the disorder, whether the polypeptide is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the polypeptide, the route of administration, the condition of the patient, and the discretion of the attending physician. The polypeptide is suitably administered to the patient at one time or over a series of treatments.

Depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (e.g., 0.1-20  $\text{mg/kg}$ ) of WISP polypeptide is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms of the disorder occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. In another

embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the WISP polypeptide. The label on, or associated with, the container indicates that the composition is used for treating the condition or disorder of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution, and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

#### F. Anti-WISP Polypeptide Antibodies

The present invention further provides anti-WISP polypeptide antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

##### 1. Polyclonal Antibodies

The anti-WISP polypeptide antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the WISP polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

##### 2. Monoclonal Antibodies

The anti-WISP polypeptide antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the WISP polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as PEG, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice (Academic Press: New York, 1986) pp. 59-103.

Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California, and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, *J. Immunol.*, **133**:3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against a WISP polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., **107**:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Goding, *supra*. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, Proc. Natl. Acad. Sci. USA, **81**:

6851-6855 (1984)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

5 The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy-chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

10 *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

### 3. Humanized Antibodies

15 The anti-WISP antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub>, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or

20 substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody preferably also will comprise at least a portion of an Fc, typically that of a human immunoglobulin. Jones *et al.*, Nature, 321:522-525 (1986); Riechmann *et al.*, Nature, 332:323-329 (1988); Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

30 Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, Nature, 321:522-525 (1986); Riechmann *et al.*, Nature, 332:323-327 (1988); Verhoeven *et al.*, Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies

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are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991).

#### 4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a WISP polypeptide; the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities. Milstein and Cuello, *Nature*, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

#### 5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection. WO 91/00360; WO 92/200373; EP 03089. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

### G. Uses for anti-WISP Polypeptide Antibodies

The antibodies of the invention may be used as affinity purification agents. In this process, the antibodies are immobilized on a solid phase such as SEPHADEX<sup>TM</sup> resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing the WISP polypeptide (or fragment thereof) to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the WISP protein, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the WISP polypeptide from the antibody.

Anti-WISP polypeptide antibodies may also be useful in diagnostic assays for WISP polypeptide, e.g., detecting its expression in specific cells, tissues, or serum. Thus, the antibodies may be used in the diagnosis of human malignancies (see, for example, U.S. Pat. No. 5,183,884).

For diagnostic applications, the antibody typically will be labeled with a detectable moiety. Numerous labels are available which can be preferably grouped into the following categories:

(a) Radioisotopes, such as <sup>35</sup>S, <sup>14</sup>C, <sup>125</sup>I, <sup>3</sup>H, and <sup>131</sup>I. The antibody can be labeled with the radioisotope using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligan *et al.*, Ed., (Wiley-Interscience: New York, 1991), for example, and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin, and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in Current Protocols in Immunology, *supra*, Coligan, ed., for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available, and U.S. Patent No. 4,275,149 provides a review of some of these. The enzyme preferably catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase,  $\beta$ -galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym., Vol. 73, Langone and Van Vunakis, eds. (New York: Academic Press, 1981), pp. 147-166.

Examples of enzyme-substrate combinations include:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxide as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (*e.g.*, orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and

5 (iii)  $\beta$ -D-galactosidase ( $\beta$ -D-Gal) with a chromogenic substrate (*e.g.*, p-nitrophenyl- $\beta$ -D-galactosidase) or fluorogenic substrate (4-methylumbelliferyl- $\beta$ -D-galactosidase).

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see, for example, U.S. Patent Nos. 4,275,149 and 4,318,980.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware  
10 of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or *vice versa*. Biotin binds selectively to avidin, and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (*e.g.*, digoxin) and one of the different types of labels mentioned above is conjugated with an  
15 anti-hapten antibody (*e.g.*, anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

In another embodiment of the invention, the anti-WISP polypeptide antibody need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the anti-WISP polypeptide antibody.

20 The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques (New York: CRC Press, Inc., 1987), pp.147-158.

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of WISP protein in the test sample is  
25 inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different  
30 immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, U.S. Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For  
35 example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tumor sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.



The antibodies may also be used for *in vivo* diagnostic assays. Preferably, the antibody is labeled with a radionuclide (such as  $^{111}\text{In}$ ,  $^{99}\text{Tc}$ ,  $^{14}\text{C}$ ,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ ,  $^{32}\text{P}$  or  $^{35}\text{S}$ ) so that the tumor can be localized using immunoscintigraphy.

5 Additionally, anti-WISP polypeptide antibodies may be useful as antagonists to WISP polypeptide functions where WISP polypeptide is upregulated in cancer cells or stimulates their proliferation or is upregulated in atherosclerotic tissue. Hence, for example, the anti-WISP polypeptide antibodies may by themselves or with a chemotherapeutic agent or other cancer treatment or drug such as anti-HER-2 antibodies be effective in treating certain forms of cancer such as breast cancer, colon cancer, lung cancer, and melanoma. Further uses for the antibodies include inhibiting the binding of a WISP polypeptide to its  
10 receptor, if applicable, or to an IGF, if applicable. For therapeutic use, the antibodies can be used in the formulations, schedules, routes, and doses indicated above under uses for the WISP polypeptides. In addition, anti-WISP polypeptide antibody may be administered into the lymph as well as the blood stream.

As a matter of convenience, the anti-WISP antibody of the present invention can be provided as an article of manufacture such as a kit. An article of manufacture containing a WISP polypeptide or antagonists  
15 thereof useful for the diagnosis or treatment of the disorders described above comprises at least a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition that is effective for diagnosing or treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection  
20 needle).

The active agent in the composition is the WISP polypeptide or an agonist or antagonist thereto. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutical: :y-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, and dextrose  
25 solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. The article of manufacture may also comprise a second or third container with another active agent as described above. A kit format generally is a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic or treatment assay.

30 If the active agent is an antibody that is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (*e.g.*, a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer), and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially maximize the sensitivity of the assay.  
35 Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia.

#### EXAMPLE 1: Isolation of cDNA Clones Encoding Mouse WISP-1

Several putative WISP genes have been identified at the mRNA level in a high-throughput PCR-select cDNA subtraction experiment carried out using a mouse mammary cell line (C57MG), which has been transformed by a Wnt-1 retroviral vector and compared with the parental cell line. The WISP family disclosed herein, including the mouse WISP-1 gene, was induced only in the transformed cell line C57MGWnt-1.

##### 1. Suppression Subtractive Hybridization

Mouse WISP-1 was isolated independently by Wnt-1 differential screening using suppression subtractive hybridization (SSH), as described by Diatchenko *et al.*, *Proc. Natl. Acad. Sci. USA*, **93**: 6025-6030 (1996). SSH was carried out using the PCR-SELECT® cDNA Subtraction Kit (Clontech Laboratories, Inc.) according to the manufacturer's protocol. Driver double-stranded (ds) cDNA was synthesized from 2 micrograms of polyA<sup>+</sup> RNA isolated from a mouse mammary cell line (C57MG), obtainable from a mouse breast cancer myoepithelial cell line. This cell line is described in Brown *et al.*, *Cell*, **46**: 1001-1009 (1986); Olson and Papkoff, *Cell Growth and Differentiation*, **5**: 197-206 (1994); Wong *et al.*, *Mol. Cell. Biol.*, **14**: 6278-6286 (1994); and Jue *et al.*, *Mol. Cell. Biol.*, **12**: 321-328 (1992), and is responsive to Wnt-1 but not to Wnt-4. Tester ds cDNA was synthesized from 2 micrograms of polyA<sup>+</sup> RNA isolated from a transformed version of C57MG, called C57MG/wnt-1.

The C57MG/wnt-1 mouse mammary derivative cell line was prepared by first transforming the parent line with a Wnt-1 retroviral vector, pBabe Puro (5.1 kb). This vector has a 5' LTR, packaging elements, a multiple cloning site, the puromycin-resistance gene driven off the SV40 promoter, a 3' LTR, and the bacterial elements for replication and ampicillin selection. The vector was modified slightly for Wnt-1 cloning by removing the *Hind*III site after the SV40 promoter and adding a *Hind*III site to the multiple cloning site. Wnt-1 is cloned from *Eco*RI-*Hind*III in the multiple cloning site. Figure 13 shows a map of the vector.

The transformed derivative cells were grown up in a conventional fashion, and the final cell population was selected in DMEM + 10% FCS with 2.5 µg/ml puromycin to stabilize the expression vector.

PCR was performed using the Clontech kit, including the cDNA synthesis primer (SEQ ID NO:40), adaptors 1 and 2 (SEQ ID NOS:41 and 42, respectively) and complementary sequences for the adaptors (SEQ ID NOS:43 and 44, respectively). PCR primer 1 (SEQ ID NO:45), PCR primer 2 (SEQ ID NO:46), nested PCR primer 1 (SEQ ID NO:47), nested PCR primer 2 (SEQ ID NO:48), control primer G3PDH5' primer (SEQ ID NO:49), and control primer G3PDH3' primer (SEQ ID NO:50), shown in Figure 14.

Products generated from the secondary PCR reaction were inserted into the cloning site region of pGEM-T vector (Promega), shown in Figure 15 (SEQ ID NOS:51 and 52 for 5' and 3' sequences, respectively). Plasmid DNAs were prepared using the WIZARD MINIPREP™ Kit (Promega). DNA sequencing of the subcloned PCR fragments was performed manually by the chain termination reaction (SEQUENASE 2.0™ Kit, Pharmacia). Nucleic acid homology searches were performed using the BLAST program noted above.

A total of 1384 clones were sequenced out of greater than 5000 found. A total of 1996 DNA templates were prepared. A program was used to trim the vector off, and a different program used to cluster the clones into two or more identical clones or with an overlap of 50 bases the same. Then a BLAST was performed of a representative clone from the cluster. Primers were designed for RT-PCR to see if the clones were differentially expressed.

## 2. Semi-quantitative RT-PCR

One of the clones was clone 568 having 71 bp, which was identified as encoding mouse WISP-1. There were six clones in this cluster. The nucleotide sequence and putative amino acid sequence of full-length mouse WISP-1 are shown in Figure 1 (SEQ ID NOS:9 and 12, respectively). RT-PCR primers were designed for confirming differential expression, screening for full-length mouse clone, and screening for the human clone. These primers were 568.PCR.top1 (nucleotides 909-932 of the full-length nucleotide sequence encoding mouse WISP-1 (SEQ ID NO:9) of Figure 1) and 568.PCR.bot1 (nucleotides 955-978 of the full-length complementary nucleotide sequence encoding mouse WISP-1 (SEQ ID NO:10) of Figure 1), which are as follows:

568.PCR.top1: 5'-CCAGCCAGAGGAGGCCACGAAC (SEQ ID NO:100)

568.PCR.bot1: 3'-TGTGCGTGGATGGCTGGGTTCATG (SEQ ID NO:101)

For the RT-PCR procedure, cell lines were grown to subconfluence before extracting the RNA. Total RNA was extracted using Stat-60™ (TEL-TEST™ B) per manufacturer's instructions. First-strand cDNA was prepared from 0.1 µg - 3 µg of total RNA with the SUPERScript™ RT kit (Gibco, BRL). PCR amplification of 5 µl of first-strand cDNA was performed in a 50-µl PCR reaction. The above primers were used to amplify first-strand cDNA. As controls, primers corresponding to nucleotide positions 707-729 (sense: 5'-GTGGCCCATGCTCTGGCAGAGGG (SEQ ID NO:102)) or 836-859 (sense: 5'-GACTGGAGCAAGGTCGTCCTCGCC (SEQ ID NO:103)) and 1048-1071 (anti-sense: 5'-GCACCACCCACAAGGAAGCCATCC (SEQ ID NO:104)) of human triosephosphate isomerase (huTPI) (Maquat *et al.*, *J. Biol. Chem.*, **260**: 3748-3753 (1985); Brown *et al.*, *Mol. Cell. Biol.*, **5**: 1694-1706 (1985)) were used to amplify first-strand cDNA. For mouse triosephosphate isomerase, primers corresponding to nucleotide positions 433-456 (sense: 5'-GACGAAAGGGAAGCCGGCATCACQ (SEQ ID NO: 105)) or 457-480 bp (sense: 5'-GAGAAGGTCGTGTTTCGAGCAAACC (SEQ ID NO: 106)) and 577-600 bp (antisense: 5'-CTTCTCGTGTAATTCCTGTGCCTG (SEQ ID NO:107)) or 694-717 bp (antisense: 5'-CACGTCAGCTGGCGTTGCCAGCTC (SEQ ID NO:108)) were used for amplification.

Briefly, 4 µCi of (<sup>32</sup>P)-CTP (3000 Ci/mmol) was added to each reaction with 2.5 U of TAKARA EX TAQ™ (Panvera, Madison, WI) and 0.2 µM of each dNTP. The reactions were amplified in a 480 PCR THERMOCYCLER™ (Perkin Elmer) using the following conditions: 94°C for 1 min., 62°C for 30 sec..

72°C for 1 min. for 18-25 cycles. 5 µl of PCR products were electrophoresed on a 6% polyacrylamide gel. The gel was exposed to film. Densitometry measurements were obtained using ALPHA EASE VERSION 3.3a™ software (Alpha Innotech Corporation) to quantitate the WISP- or TPI-specific gene products.

### 3. Northern Blot Analysis

5 Adult multiple-tissue Northern blots (Clontech) and the Northern blot of the C57MG parent and C57MG/Wnt-1 derivative polyA+RNA (2 µg/lane) were hybridized with a 70-bp mouse WISP-1 probe (amino acids 278 through 300 of Fig. 1; QPEEATNFTLAGCVSTRTYRPKY; SEQ ID NO:109) generated using the primers 568.PCR.top1 and 568.pcr.bot1 noted above. The membranes were washed in 0.1 X SSC at 55-65°C and exposed for autoradiography. Blots were rehybridized with a 75-bp synthetic probe from the  
10 human actin gene. See Godowski *et al.*, Proc. Natl. Acad. Sci. USA, **86**: 8083-8087 (1989) for a method for making a probe with overlapping oligos, which is how the actin probe was prepared.

### 4. cDNA Library Screening

Clones encoding the full-length mouse WISP-1 were isolated by screening a λgt10 oligodT primed mouse embryo library (Clontech) with the primers 568.PCR.top1 and 568.PCR.bot1 noted above. The inserts  
15 of 13 of these clones were subcloned into pBLUESCRIPT™ IISK+ and their DNA sequences determined by dideoxy DNA sequencing on both strands.

### 5. Results

The recently described technique of SSH combines a high subtraction efficiency with an equalized representation of differentially expressed sequences. This method is based on specific PCR reactions that  
20 permit exponential amplification of cDNAs which differ in abundance, whereas amplification of sequences of identical abundance in two populations is suppressed. The SSH technique was used herein to isolate genes expressed in a mouse mammary myoepithelial cell transformed with *Wnt-1* whose expression is reduced or absent in the parental myoepithelial cell. The polyA+RNA extracted from both types of cells was used to synthesize tester and driver cDNAs. The degree of subtraction efficiency was monitored by Southern blot  
25 analysis of unsubtracted and subtracted PCR products using a β-actin probe. No β-actin mRNA was apparent in the subtracted PCR products, confirming the efficiency of the subtraction.

The subtracted cDNA library was subcloned into a pGEM-T vector for further analysis. A random sample of 1996 clones was sequenced from the transformed colonies obtained. To determine if the clones  
30 obtained were differentially expressed, PCR primers were designed for selected clones and semi-quantitative RT-PCR and Northern analyses were performed using mRNA from the mouse mammary cell line and its derivative. It was found that expression of *Wnt-1* in C57MG cells leads to elongated cell morphology and loss of contact inhibition.

One clone (m568.19A) of those that fulfilled the criteria for differential expression was found to  
35 encode full-length mouse WISP-1. By both RT-PCR analysis and Northern analysis, it was found that this clone provided an about three-fold induction in the Wnt-1 cell line over the parent cell line.

The cDNA sequence of this clone and deduced amino acid sequence of full-length mouse WISP-1 are shown in Figure 1 (SEQ ID NOS:9 and 12, respectively). The sequence alignment of human and mouse WISP-1 (SEQ ID NOS:4 and 12, respectively) is shown in Figure 8. *In-situ* analysis of the clone is presented below, along with thymidine incorporation assay and angiostatic assay results.

This clone was placed in pRK5E, an *E. coli*-derived cloning vector having a human cytomegalovirus intermediate early gene promoter, an SV40 origin and polyA site, an sp6 transcription initiation site, a human immunoglobulin splice acceptor, and *XhoI*/*NotI* cDNA cloning sites. It is a progeny of pRK5D that has an added *SceI* site. Holmes *et al.*, Science, 253:1278-1280 (1991). Upon transformation into JM109 cells, the plasmid rendered the cells ampicillin resistant. Upon digestion with *XbaI* and *Bam*HI, a 1140-bp fragment was obtained, and the mouse insert size was 1122 base pairs, from the ATG to the stop codon, including a 3' tag of six histidines.

#### EXAMPLE 2: Isolation of a cDNA Clone Encoding Mouse WISP-2

The cDNA for mouse WISP-2 was isolated independently by Wnt-1 differential screening using the procedure described in Example 1. The initial clone isolated was 318 bp in length and was designated clone 1367. There were four clones in this cluster. The clone was sequenced as described above and RT-PCR primers were designed as follows:

1367.pcr.top1: nucleotides 1604-1627 of Figure 2:

3'-GGTGTGAAGACCGTCCGGTCCCGG (SEQ ID NO:110)

and

1367.pcr.bot1: nucleotides 1438-1461 of Figure 2:

5'-GTGTGCCTTTCCTGATCTGAGAAC (SEQ ID NO:111)

After RT-PCR and Northern blot procedures were carried out as described in Example 1 to confirm differential expression, a five-fold induction in the Wnt-1 cell line was observed.

Clones encoding full-length mouse WISP-2 were isolated from RNA library 211: C57MG/Wnt-1. mRNA for construction of this library was isolated from the C57MG/Wnt-1 cell line described in Example 1. The RNA was used to generate an oligo-dT-primed cDNA library in the cloning vector pRK5E using reagents and protocols from Life Technologies, Gaithersburg, MD (SUPERSCRIPT PLASMID SYSTEM<sup>TM</sup>).

In this procedure, the double-stranded cDNA was primed with oligo dT containing a *NotI* site, linked with blunt-to-*Sall* hemikinased adaptors, cleaved with *NotI*, sized to greater than 1000 bp appropriately by gel electrophoresis, and cloned in a defined orientation into the *XhoI*/*NotI*-cleaved pRK5E vector. The library was screened by colony hybridization with a probe 1367.50mer.1 of bases 1463-1512 of Figure 2:

3'-GGGACGGGCCGACCCTTCTTAAAAGACCCTTGTACTTCTCTACCTTAGTG (SEQ ID NO:112).

The full-length mouse WISP-2 clone was obtained, designated clone 1367.3.

The cDNA for mouse WISP-2, like the mouse WISP-1 molecule, encodes a novel secreted protein that belongs to the CTGF family and is the mouse homologue of SST DNA33473 of Example 4. (The alignment of human and mouse WISP-2 (SEQ ID NOS:16 and 20, respectively) is shown in Figure 9.) The mouse WISP-2 gene is 38% identical in sequence to mouse WISP-1, disclosed in Example 1, but lacks the C-terminal 95 amino acids thought to be involved in dimerization and receptor binding. Mouse WISP-2 was highly expressed in the lung. *In-situ* analysis of the clone is noted below. The nucleotide sequence and putative amino acid sequence of full-length mouse WISP-2 are shown in Figure 2 (SEQ ID NOS:17 and 20, respectively). The putative signal sequence is from amino acid positions 1 to 23 of SEQ ID:20.

The clone was inserted into pRK5E, described above. Upon transformation of JM109 cells, the plasmid rendered the cells ampicillin resistant. Upon digestion with *Bam*HI and *Nor*I, a 1770-bp fragment was obtained, having a mouse insert of 756 bp from ATG to the stop codon.

EXAMPLE 3: Isolation of a cDNA Clone Encoding Human WISP-1

To isolate the full-length human clone corresponding to m568.19A (mouse WISP-1), a human lung cDNA library (Clontech), treated with the SUPERSCRIP<sup>TM</sup> kit using the pRK5E vector as described above, was screened with a 70-bp probe at low stringency (20% formamide, 1 X SSC, 55°C wash). The probe had the sequence from nucleotides 909-978 of the full-length mouse WISP-1 nucleotide sequence of Figure 1, *i.e.*, the sequence:

5'-CCAGCCAGAGGAGGCCACGAACTTCACTCTCGCAGGCTGTGTCAGCACACGCACCTACC  
GACCCAAGTAC (SEQ ID NO:113)

Only one clone was identified, hL.568.15A. The insert to this clone was subcloned into pBLUESCRIPT<sup>TM</sup> IISK+ and its DNA sequence determined by dideoxy DNA sequencing on both strands. This clone was found to be missing about 280 amino acids. Hence, a new probe (hu.568.50mer.1) was designed from clone 15A having the nucleotides 750-799 of the full-length human WISP-1 nucleotide sequence shown in Figures 3A and 3B, *i.e.*,

5'-GCCCCCTGGAGCCCTTGCTCCACCAGCTGCGGCCTGGGGGTCTCCACTCGG (SEQ ID NO:114)

This probe was used to screen a human fetal kidney cDNA library (Clontech), treated with the SUPERSCRIP<sup>TM</sup> kit using the pRK5E vector as described above, by colony hybridization. A number of clones were obtained by screening this human fetal kidney cDNA library (clones without the A or B designation) or by screening a human fetal kidney λgt10 library (clones with the A or B designation) using the same probes described above. The inserts of these clones were subcloned into pBLUESCRIPT<sup>TM</sup> IISK+ and their DNA sequences determined by dideoxy DNA sequencing on both strands.

Two of these clones, designated as 568.1A and 568.4A, have their respective sequences (SEQ ID NOS:24 and 26) shown in Figures 27 and 29. These clones are missing the von Willebrand C1 domain, the variable domain, and the thrombospondin 1 domain, and have a frameshift. Other clones, designated as 568.13, 568.39, 568.5A, 568.6B, and 568.7 (SEQ ID NOS:23, 25, 27, 28, and 29, respectively; Figs. 26, 28, and 30-32, respectively), were obtained that lack one or more domains and/or short amino-acid stretches (*e.g.*, an 8- amino-acid deletion) or contain additional short amino-acid stretches and may contain introns or alternative splice variants.

Two clones sharing a significant amount of sequence with the full-length clone 568.38 were identified: 568.23 and 568.35. Human clone 568.38 encoded the full-length human WISP-1. The nucleotide sequence and putative amino acid sequence for clone 568.38 are shown in Figures 3A and 3B (SEQ ID NOS:1 and 4, respectively). The aligning sequences of clones 568.38 and 568.35 differ from the corresponding aligning sequences of clones 568.15A and 568.23 in that the respective sequences of the latter two clones have an isoleucine residue at amino acid position 184 of Figs. 3A and 3B, whereas the respective corresponding sequences of clones 568.38 and 568.35 have a valine residue at this position. Further, the aligning sequences of clones 568.35 and 568.38 differ from each other in that the sequence of clone 568.35

has a serine residue at amino acid position 202 of Figs. 3A and 3B, whereas the corresponding sequence of clone 568.38 has an alanine residue at this position.

The human WISP-1 polypeptide, by homology searching, is also found to be a member of the CTGF family. The clone was placed in a pRK5E plasmid as described above and deposited with the ATCC. Upon transformation into JM109 cells, the plasmid rendered the cells ampicillin resistant. Digestion with *Clal* and *EcoRV* yielded a 1435-bp fragment with an insert size of 1104 basepairs from ATG to the stop codon.

*In situ* hybridization of human WISP-1 was performed, with the results given below. Northern analysis of human WISP-1 showed high expression in adult heart tissue and ovary tissue, and in fetal kidney tissue. Also presented below are thymidine incorporation assay, gene amplification assay, and angiostatic assay results.

The chromosomal location of the human WISP genes was determined by radiation hybrid mapping using the Stanford G3<sup>TM</sup> and the MIT Genebridge 4 Radiation Hybrid<sup>TM</sup> panels. WISP-1 resides at approximately 3.48 cR from the meiotic marker AFM259xc5 (LOD score 16.31) on the Genebridge map. This places WISP-1 in band 8q24.1 to 8q24.3, roughly four megabases distal to *c-myc* located at chromosome band 8q24.12-8q24.13. Takahashi *et al.*, *Cytogenet. Cell Genet.*, 57: 109-111 (1991). *c-myc* is a region that is a recurrent site of amplification in non-small cell lung carcinoma.

#### EXAMPLE 4: Isolation of a cDNA Clone Encoding Human PRO261 (designated herein as human WISP-2)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the SWISS-PROT<sup>TM</sup> public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (*e.g.*, GenBank) and a proprietary EST DNA database (LIFESEQ<sup>TM</sup>, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6-frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington: <http://bozeman.mbt.washington.edu/phrap.docs/phrap.html>).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. The EST sequences used (from Incyte) were Nos. 2633736, 2118874, 360014, 2316216, 1985573, 2599326, 1544634, 2659601, 1319684, 783649, 627240, 1962606, 2369125, 939761, 1666205, 692911, 984510, 1985843, 2104709, and 2120142. This consensus sequence is herein designated DNA30843 (see Fig. 5). Based on the DNA30843 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO261 (human WISP-2). A pair of PCR primers, forward and reverse, were synthesized having the respective sequences:

5'-AAAGGTGCGTACCCAGCTGTGCC (SEQ ID NO:115) and  
3'-TCCAGTCGGCAGAAGCGGTTCTGG (SEQ ID NO:116).

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30843 sequence, which probe has the sequence:

5'-CCTGGTGCTGGATGGCTGTGGCTGCTGCCGGGTATGTGCACGGCGGCTGGG (SEQ ID NO:117).

For screening several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, Current Protocols in Molecular Biology (Green Publishing Associates and Wiley Interscience, N.Y., 1989), with the PCR primer pair identified above. A positive library was then screened by colony hybridization to isolate clones encoding PRO261 (human WISP-2) using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a *NotI* site, linked with blunt-to-*SacI*-hemikinased adaptors, cleaved with *NotI*, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRK5B or pRK5D; pRK5B is a precursor of pRK5D that does not contain the *SfiI* site; see Holmes *et al.*, Science, 253:1278-1280 (1991)) in the unique *XhoI* and *NotI* sites.

DNA sequencing of the clones isolated as described above gave the DNA sequence for PRO261 (herein designated as UNQ228 (DNA33473-seq min); SEQ ID NO:38), which begins at nucleotide 13 of SEQ ID NO:13 (Fig. 4) and the derived amino acid sequence for PRO261 (SEQ ID NO:16).

The entire nucleotide sequence encoding human WISP-2 is shown in Figure 4 (SEQ ID NO:13). This sequence contains a single open reading frame with an apparent translational initiation site at nucleotide positions 22-24 of SEQ ID NO:13 and ending at the stop codon after nucleotide 770 of SEQ ID NO:13 (Figure 4). The predicted polypeptide precursor is 250 amino acids long (Figure 4). The putative signal sequence spans from amino acid positions 1 to 23 of SEQ ID NO:16. Clone UNQ228 (DNA33473-seq min) has been deposited with ATCC and is assigned ATCC deposit no. 209391.

Analysis of the amino acid sequence of the full-length PRO261 polypeptide suggests that portions of it possess significant homology to CTGF, thereby indicating that PRO261 is a novel growth factor.

*In situ* hybridization of human WISP-2 is given below. The chromosomal location of the human WISP-2 gene was determined as described above for human WISP-1. Specifically, WISP-2 is linked to the marker SHGC-33922, with a LOD score of 1000. This places WISP-2 in band 20q12-20q13.1. Human chromosome 20q12 is a frequent site of DNA amplification in human breast cancer. In a *Xenopus* assay, injection of human WISP-2 RNA partially induced axis duplication (see Example 11). Also presented below are thymidine incorporation assay, gene amplification assay, and angiostatic assay results for human WISP-2.

#### EXAMPLE 5: Isolation of cDNA Clones Encoding Human WISP-3

In this example, the gene encoding WISP-3 was cloned twice essentially in parallel. First, it was determined whether the databases described above contained any new members of the WISP family. Two EST homologies to the WISPs were found and both were cloned. Full-length clones were isolated corresponding to each of these EST homologies. The efforts resulted in two full-length clones of the same gene (the original EST homologies had been from distinct regions of the same gene). The first clone obtained was designated as DNA56350 and the second as DNA58800.



DNA56350

Based on the sequence of INCYTE 3208053, a virtual DNA 48917 was obtained and oligonucleotides were synthesized for use as probes to isolate a clone of the full-length coding sequence for PRO956 (human WISP-3). A pair of PCR primers, forward and reverse, were synthesized having the sequences:

5'-GTCTTGTGCAAGCAACAAAATGGACTCC (SEQ ID NO:118)

3'-GACACAATGTAAGTCGGAACGCTGTCTG (SEQ ID NO:119)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the INCYTE sequence, which probe has the sequence:

5'-GCTCCAGAACATGTGGGATGGGAATATCTAACAGGGTGACCAATGAAAQ (SEQ ID NO:120)

A human fetal kidney library primed with oligo dT containing a *XhoI-NotI* size cut greater than 3700 kb was screened for a source of a full-length clone by PCR amplification with the PCR primer pair identified above. The positive library was then used to isolate clones encoding PRO956 (human WISP-3) using the probe oligonucleotide and one of the PCR primers.

DNA sequencing of the clone isolated as described above gave the DNA sequence for PRO956 (herein designated as UNQ462 (SEQ ID NO:30), and the derived amino acid sequence for PRO956 (SEQ ID NO: 33).

The entire nucleotide sequence encoding human WISP-3 from this clone is shown in Figure 6 (SEQ ID NO:30). This sequence contains a single open reading frame with an apparent translational initiation site at nucleotide positions 46-48 of SEQ ID NO:30 and ending at the stop codon after nucleotide 1161 of SEQ ID NO:30 (Figure 6). The predicted polypeptide precursor is 372 amino acids long (Figure 6). The putative signal sequence is from amino acid positions 1 to 33 of SEQ ID NO:33. Clone UNQ462 (DNA56350-1176-2) has been deposited with ATCC and is assigned ATCC deposit no. 209706.

Analysis of the amino acid sequence of the full-length PRO956 polypeptide suggests that portions of it possess significant homology to CTGF, thereby indicating that PRO956 is a novel growth factor. This clone has an additional methionine just 5' of the first methionine in this clone. The amino acid sequence of this clone is 42% homologous to that of human WISP-1, and 32% homologous to that of human WISP-2.

*In situ* hybridization of human WISP-3 is shown below. Using the mapping techniques set forth above, it was found that human WISP-3 was localized to chromosome 6q22-6q23 and was linked to the marker AFM211ze5 with a LOD score of 1000. WISP-3 is approximately 18 megabases proximal to CTGF and 23 megabases proximal to the human cellular oncogene MYB, which are also located at 6q22-6q23. Martinerie *et al.*, Oncogene, 7: 2529-2534 (1992); Meese *et al.*, Genes Chromosomes Cancer, 1: 88-94 (1989).

The clone was inserted into pRK5E, described above. Upon transformation of JM109 cells, the plasmid rendered the cells ampicillin resistant. Upon digestion with *Bam*HI and *Not*I, a fragment was obtained having a human insert from ATG to the stop codon as indicated in Figure 6.

DNA58800

Based on the sequence of HS142L7, a virtual DNA 56506 was obtained and oligonucleotides were synthesized for use as probes to isolate a clone of the full-length coding sequence for PRO790 (human WISP-3). To this end, a pair of PCR primers, forward and reverse, were synthesized having the sequences:

5'-CCTGGAGTGAGCCTGGTGAGAGA (SEQ ID NO:121)

3'-ACACTGGGTGTGTTCCCGACATAACA (SEQ ID NO:122)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the HS142L7 sequence, which probe has the sequence:

5'-TGGTTGCTTGGCACAGATTTTACAGCATCCACAGCCATCTCTCA (SEQ ID NO:123)

A human bone marrow library primed with oligo dT containing a *XhoI-NotI* size cut of 1-3 kb was screened for a source of a full-length clone by PCR amplification with the PCR primer pair identified above. The positive library was then used to isolate clones encoding PRO790 (human WISP-3) using the probe oligonucleotide and one of the PCR primers.

DNA sequencing of the clone isolated as described above gave the DNA sequence for PRO790 (SEQ ID NO:34), and the derived amino acid sequence for PRO790 (SEQ ID NO:37).

The entire nucleotide sequence encoding human WISP-3 from this clone is shown in Figure 7 (SEQ ID NO:34). This sequence contains a single open reading frame with an apparent translational initiation site at nucleotide positions 16-18 of SEQ ID NO:34 and ending at the stop codon after nucleotide 1077 of SEQ ID NO:34 (Figure 7). The predicted polypeptide precursor is 355 amino acids long (Figure 7). The putative signal sequence spans from amino acid positions 1 to 15 of SEQ ID NO:37. This clone DNA58800-1176-2 has been deposited with ATCC and is assigned ATCC deposit no. 209707.

Analysis of the amino acid sequence of the full-length PRO790 polypeptide suggests that portions of it possess significant homology to CTGF, thereby indicating that, like PRO956 which is a splice variant thereof, PRO790 is a novel growth factor.

*In situ* hybridization of human WISP-3 is shown below.

The clone was inserted into pRK5E, described above. Upon transformation of JM109 cells, the plasmid rendered the cells ampicillin resistant. Upon digestion with *BamHI* and *NotI*, a fragment was obtained having a human insert from ATG to the stop codon as indicated in Figure 7.

EXAMPLE 6: Use of WISP-Encoding DNA as a Hybridization Probe

The following method describes use of a nucleotide sequence encoding a WISP polypeptide as a hybridization probe.

DNA comprising the coding sequence of full-length or mature human WISP-1 (as shown in Figures 3A and 3B, SEQ ID NOS:4 or 3, respectively), or full-length or mature mouse WISP-1 (as shown in Figure 1, SEQ ID NOS:12 or 11, respectively), or of full-length or putative mature human WISP-2 (as shown in Fig. 4, SEQ ID NOS:16 or 15, respectively), or full-length or putative mature mouse WISP-2 (as shown in Figure 2, SEQ ID NOS:20 or 19, respectively) is employed as a probe to screen for homologous DNAs (such as those encoding naturally occurring variants of these particular WISP proteins in human tissue cDNA libraries or human tissue genomic libraries).

Hybridization and washing of filters containing either library DNAs is performed under the following high-stringency conditions. Hybridization of radiolabeled WISP-polypeptide-derived probe (such as UNQ228 (DNA33473-seqmin)-derived probe) to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding a full-length, native-sequence WISP polypeptide can then be identified using standard techniques known in the art.

#### EXAMPLE 7: Expression of WISP Polypeptide in *E. coli*

This example illustrates preparation of an unglycosylated form of WISP polypeptide by recombinant expression in *E. coli*.

The DNA sequence encoding WISP polypeptide is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, Gene, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR-amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode an antibiotic-resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the WISP-coding region, lambda transcriptional terminator, and an *argU* gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates, and antibiotic-resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger-scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After the cells are cultured for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the WISP polypeptide can then be purified using a metal-chelating column under conditions that allow tight binding of the protein.

#### EXAMPLE 8: Expression of WISP Polypeptide in Mammalian Cells

This example illustrates preparation of a potentially glycosylated form of WISP polypeptide by recombinant expression in mammalian cells.

The vector, pRK5E, was employed as the expression vector. The appropriate DNA encoding WISP polypeptide was ligated into pRK5E with selected restriction enzymes to allow insertion of the DNA for WISP polypeptide using ligation methods as described in Sambrook *et al.*, *supra*. The resulting vectors were pRK5E.h.WIG-1.568.38, pRK5E.m.WIG-1.568.6his, pRK5E.m.WIG-2.1367.3, plasmid encoding human

WISP-2, DNA56350-1176-2, and DNA58800-1176-2, all deposited at the ATCC. These vectors are conveniently referred to collectively as pRK5E.WISP in the general description below.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5E.WISP DNA is mixed with about 1 µg DNA encoding the VA RNA gene (Thimmappaya *et al.*, Cell, 31:543 (1982)) and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in phosphate-buffered saline (PBS) is added for 30 seconds. The 293 cells are then washed with serum-free medium, fresh medium is added, and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine and 200 µCi/ml <sup>35</sup>S-methionine. After a 12-hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of the WISP polypeptide. The cultures containing transfected cells may undergo further incubation (in serum-free medium) and the medium is tested in selected bioassays.

In an alternative technique, the WISP polypeptide may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac *et al.*, Proc. Natl. Acad. Sci., 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5E.WISP DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin, and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media are centrifuged and filtered to remove cells and debris. The sample containing expressed WISP polypeptide can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, the WISP polypeptide can be expressed in CHO cells. The pRK5E.WISP can be transfected into CHO cells using known reagents such as CaPO<sub>4</sub> or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as <sup>35</sup>S-methionine. After determining the presence of the WISP polypeptide, the culture medium may be replaced with serum-free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed WISP polypeptide can then be concentrated and purified by any selected method.

Epitope-tagged WISP polypeptide may also be expressed in host CHO cells. The WISP polypeptide may be subcloned out of the pRK5 vector. Suva *et al.*, Science, 237: 893-896 (1987); EP 307,247 published 3/15/89. The subclone insert can undergo PCR to fuse in-frame with a selected epitope tag such as a poly-his tag into a baculovirus expression vector. The poly-his-tagged WISP polypeptide insert can then be subcloned into a SV40-driven vector containing a selection marker such as DHFR for selection of stable clones. Finally,

the CHO cells can be transfected (as described above) with the SV40-driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His-tagged WISP can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

5 In particular, mouse WISP-1 cDNA for insertion into mammalian expression vectors was created via PCR using clone m568.19A (see above) pure phage DNA as template and using as primers m.568.pcr.top4 (5'-TGACTTCCAGGCATGAGGTGGCTCCTG; SEQ ID NO:124) and m.568.pcr.bot3 (5'-ATTGGCAATCTCTTCGAAGTCAGGGTAAGATTCC; SEQ ID NO:125) for the 6-his version, or  
10 m.568.pcr.top4 (SEQ ID NO:124) and 568.pcr.bot5, which has a 3'-terminal *Xba*I site (5'-GGTACGTCTAGACTAATTGGCAATCTCTTCGAAGTCAGGG; SEQ ID NO:126) for the non-his version. The insert integrity was confirmed by sequencing and analyzed. The PCR was run using *Pfu* polymerase and the conditions were:

	<u>temp.</u>	<u>time</u>
denaturation	94°C	1 min
15 annealing	62°C	30 sec
extension	72°C	1.5 min
# of cycles: 25		

For transient expression in 293 cells analyzed by Western blot, the above inserts were ligated into the pRK5 vector referred to above at the *Bam*HI/*Xba*I site using the BOEHRINGER MANNHEIM<sup>TM</sup> rapid  
20 ligation kit. The resulting plasmids were designated pRK5.mu.WISP-1.6his and pRK5.mu.WISP-1.nohis for the His-tagged and non-His-tagged inserts, respectively. Then the 293 cells were plated and allowed to reach approximately 85% confluency overnight (37°C/5%  $\text{CO}_2$ ). The plated cells were transfected with plasmid DNA pRK5.mu.WISP-1.6his or pRK5.mu.WISP-1.nohis by using lipofectamine (Gibco BRL) at a 4.5:1 lipid:DNA ratio.

25 Transfection efficiency (>70% usually) was monitored using a GFP expression plasmid (pGREEN LANTERN<sup>TM</sup> from Gibco BRL). Approximately 5 hours post-transfection, the medium was changed to fresh SF media (50:50 with 1X L-Glu and 1X P/S) for protein production. The conditioned media was allowed to accumulate for specified amounts of time (depending on the experiment) before harvesting.

The medium was harvested and concentrated in the presence of 1 mM PMSF using the  
30 CENTRICON-10<sup>TM</sup> concentrator. The concentrated, conditioned media (usually 1.5 ml) was then bound to  $\text{Ni}^{++}$ NTA agarose beads (Qiagen) for 2 hours (for the His-tagged version only). Protein was eluted from the beads by boiling for 10 minutes in 2X SDS loading buffer (Novex) with or without beta-mercaptoethanol for reduced vs. non-reduced protein, respectively.

The protein was visualized by SDS-PAGE using 4-20% gradient TRIS-glycine gels, 10-wells, 1 mm  
35 thickness (Novex). Gels ran at 125 volts (constant) for 95 minutes. Western transfer was achieved using a NOVEX<sup>TM</sup> transfer apparatus to PVDF membranes (Novex) at 200 mA (constant) for 45 minutes. The blots were blocked for a minimum of 1 hour at room temperature in blocking buffer (PBS + TWEEN-20<sup>TM</sup> (0.5%), 5% dry milk, and 3% goat serum). Blots were incubated in primary antibody (for His-tagged protein: INVITROGEN<sup>TM</sup> anti-his(C-terminal)-HRP-conjugated antibody or for the non-His version: polyclonal anti-

murine WISP-1 antibody prepared as described below) at a 1:2000 dilution in fresh blocking buffer for 1 hour at room temperature. The non-His-tagged protein blots were incubated in secondary antibody (PIERCE<sup>TM</sup> goat anti-rabbit IgG(H+L) HRP conjugated) diluted 1:2000 in fresh blocking buffer. The blots were incubated in chemiluminescent substrate (ECL<sup>TM</sup> from Amersham or SUPERSIGNAL<sup>TM</sup> from Pierce) for 1 minute before exposing to film.

For transient expression analyzed by antibody staining, 293 cells were cultured, plated, and transfected as described above. The cells were fixed to culture dishes for 2 minutes in 1:1 methanol:acetone solution. Fixed cells were then incubated in primary antibody (for His-tagged protein: INVITROGEN<sup>TM</sup> anti-his(C-term)-HRP-conjugated antibody or for the non-His version: polyclonal anti-murine WISP-1 antibody prepared as described below) diluted 1:1000 in PBS with 10% fetal bovine serum for 2 hours. The non-His-tagged protein blots were then incubated in secondary antibody (PIERCE<sup>TM</sup> goat anti-rabbit IgG(H+L) HRP conjugated) diluted 1:150 in PBS with 10% fetal bovine serum for 1 hour. The incubation was in color reagent substrate for HRP for up to 1 hour (1.0% O-dianisidine-saturated ETOH, 0.01% hydrogen peroxide in PBS).

For stable expression of mouse WISP-1 in mammalian cells, the starting vector employed was pRK5.CMV.puro-dhfr, the sequence of which is shown in Figures 16A-16D. This vector has two SAR sequences cloned into *KpnI*, *SapI* sites of the SVID5 splice-donor vector, and has the pSV1 backbone with the pRK5 cloning linker (pSV15) and the intron made from pSV1.WTSD.D by adding a linearization linker (LL) into the *HpaI* site. The sequence is edited to include changes in vector puc118 backbone derived from the sequence of pRK5 and includes a four-base insertion after MCS characteristic of the SV1 vector.

The above inserts were ligated into pRK5.CMV.puro-dhfr at the *BamHI/XbaI* site using the BOEHRINGER MANNHEIM<sup>TM</sup> rapid ligation kit, producing pRK5.CMV.puro-dhfr.mu.WISP-1.6his or pRK5.CMV.puro-dhfr.mu.WISP-1.nohis. This construct allows for stable selection of expressing cells using either puromycin (2 µg/ml in 293 cells or 10 µg/ml in CHO-DP12 cells) or the <sup>+</sup>HFR deletion in the CHO-DP12 line, which allows for subsequent amplification in methotrexate. Isolated colonies representative of stably transfected cells were picked, cultured under selective pressure, and analyzed by antibody staining or Western blot as described above.

#### EXAMPLE 9: Expression of WISP Polypeptide in Yeast

The following method describes recombinant expression of a WISP polypeptide in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of a WISP polypeptide from the ADH2/GAPDH promoter. DNA encoding a WISP polypeptide and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression. For secretion, DNA encoding a WISP polypeptide can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native WISP signal peptide or other mammalian signal peptide or yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be

analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant WISP polypeptide can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing the WISP polypeptide may further be purified using selected column chromatography resins.

EXAMPLE 10: Expression of WISP Polypeptide in Baculovirus-Infected Insect Cells and Purification Thereof

The following method describes recombinant expression of a WISP polypeptide in baculovirus-infected insect cells, and purification thereof.

General

The sequence coding for WISP polypeptide is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding WISP polypeptide or the desired portion of the coding sequence (such as the sequence encoding the mature protein if the protein is extracellular) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BACULOGOLD™ virus DNA (PharMingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from Gibco-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, Baculovirus Expression Vectors: A Laboratory Manual (Oxford: Oxford University Press, 1994).

Expressed poly-His-tagged WISP polypeptide can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, Nature, **362**:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL HEPES, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8), and filtered through a 0.45 µm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water, and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes non-specifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM imidazole gradient in the secondary wash buffer. One-mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen).

Fractions containing the eluted His<sub>10</sub>-tagged WISP polypeptide are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG-tagged (or Fc-tagged) WISP polypeptide can be performed using known chromatography techniques, including, for instance, Protein A or protein G column chromatography.

### Specific

#### 1. Expression

In particular, mouse WISP-1 polypeptide was expressed in a baculovirus expression system similar to that described above using as the baculovirus transfer vector pb.PH.mu.568.9.IgG.baculo or pbPH.mu.568.8his.baculo. Figures 17A-17D show the sequence (SEQ ID NO:54) of plasmid pb.PH.IgG, which was used to prepare pb.PH.mu.568.9.IgG.baculo. Figures 18A-18D show the sequence (SEQ ID NO:55) of plasmid pbPH.His.c. which was used to prepare pbPH.mu.568.8his.baculo.

Both of these baculovirus transfer vectors are based on pVL1393 (PharMingen), which has neither the His nor Fc tags. The pb.PH.IgG vector (Fig. 17) allows the expression of foreign proteins under control of the AcNPV polyhedrin promoter, which is active in the very late phase of virus infection. The foreign protein can be expressed as a C-terminal human IgG fusion protein. The His(8)-tag will not be translated as a result of the IgG stop codon just 5' of the His(8)-tag. The sequence encoding the foreign protein should be inserted as a 3' blunt-ended fragment into the unique *StuI* site preceding the His-tag. In that case an additional proline residue will be added. The 5' site can be either *Bam*HI, *Eco*RI, *Not*I, *Nco*I, and *Nhe*I.

The IgG vector was constructed by *Nde*I digestion of the pVL1393.IgG plasmid followed by Klenow treatment to fill in the sticky end site. This is followed by a *Nco*I digest and insertion into the pbPH.His.c x *Nco*I/*Sma*I-digested vector.

The sequence of pbPH.His.c shown in Figs. 18A-18D contains the backbone sequence of the vector pVL1392, which contains approximately the *Eco*RI/*Xma*III fragment of AcMNPV C-6, from position 0.0 to 5.7 mu. Possee *et al.*, *Virology*, 185: 229-241 (1991). It allows the expression of foreign proteins under control of the *Autographa californica* nuclear polyhedrosis virus (AcNPV) polyhedrin gene promoter, which is active in the very late phase of virus infection.

The foreign protein can be expressed as a C-terminally His- or a IgG (Fc region only)-tagged protein. The sequence encoding the foreign protein should be inserted as a 3'-blunt-ended fragment into the unique *Sma*I site preceding the His-tag or the *Stu*I site for IgG. In that case an additional glycine residue will be added for His tags and a proline will be added for IgG tags. The 5' site can be either *Bam*HI, *Not*I, *Eco*RI, or *Nco*I. *Bam* HI was used for both.

The vectors were constructed by inserting a PCR insert into *Bam*HI/*Sma*I for the His vector and *Bam*HI/*Stu*I for the IgG vector. The PCR insert was made using 5'-phosphorylated primers as follows: m.568.pcr.top6 (5'-TTTCCCTTTGGATCCTAAACCAACATGAGGTGGCTCCTGCCC; SEQ ID NO:127) and m.568.pcr.bot3 (SEQ ID NO:125), 5' phosphorylated. A twenty-cycle PCR reaction with Pfu polymerase enzyme was performed using the following conditions: 1 min at 95°C, 30 sec at 60°C, 3.5 min at 72°C. The PCR product was purified with QIAQUICK<sup>TM</sup> and digested with *Bam*HI at 37°C for 1 hr. The digested PCR insert was ligated into the digested vector using a 1:3 ratio of insert to vector with 1 µl T4 DNA ligase (Bio



Labs). ULTRA MAX<sup>TM</sup> DH5a FT competent cells, 100  $\mu$ l. (Gibco BRL Cat #10643-013) were added to the ligation product, and the mixture was incubated on ice for 30 min. followed by a heat shock at 42°C for 45 sec. Individual colonies were picked and miniscreen DNA was prepared using QIA PREP<sup>TM</sup> (Qiagen). Construct sequencing was performed using ABI Prism's dRHODAMINE DYE<sup>TM</sup> terminator cycle sequencing.

The plasmid pb.PH.IgG has a polylinker *Bam*HI-*Not*I-*Eco*RI-*Nco*I-*Srf*I-*Stu*I-(IgG Fc region only)-*Stop*-*Xba*I-*Spe*I-*Pst*I-*Bgl*II. The location of particular regions in this plasmid is as follows: Insertion of polylinker/foreign gene: 4129-4912 (*Bam*HI-*Bgl*II), polh coding: 4913-5479, ORF 1629: 7134-4820; ORF 588 (PK1): 7133-7723; ColEI origin of replication: 7973-8858, and ampicillin coding: 9779-8230. The plasmid pbPH.His.c has a polylinker *Bam*HI-*Not*I-*Eco*RI-*Nco*I-*Srf*I-*Sma*I-(His8)-*Stop*-*Xba*I-*Spe*I-*Pst*I-*Bgl*II. The *Nco*I site of pbPH.His.c resides within a Kozak sequence. The location of particular regions in this plasmid is as follows: ORF 504 (PTP): 76-582, ORF 984 (ORF2): 1600-614, ORF 453 (ORF3): 2323-1868, conotoxin: 1818-1657, ORF 327 (ORF4): 2352-2681, ORF 630 (lef-2): 2662-3294, ORF 603: 3937-3332, ORF polh: 4093 (mutated codon ATG/ATT), insertion of polylinker/foreign gene: 4129-4218 (*Bam*HI-*Bgl*II), polh coding: 4224-4790, ORF 1629: 6445-4820, ORF 588 (PK1): 6444-7034, ColEI origin of replication: 7284-8169, and ampicillin coding: 9090-8230.

The mouse WISP-1 cDNA disclosed herein was inserted into the vectors pbPH.His.c and pb.PH.IgG to produce the respective expression plasmids by creating a 3' blunt-ended fragment for cloning into the unique *Sma*I site preceding the His-tag or IgG-tag. An additional glycine residue was added to the His protein produced. An additional proline was added to the IgG protein. The 5' site of the cDNA insert was *Bam*HI.

## 2. Purification

For purification purposes, either a poly-His tag or the Fc portion of human IgG was added to the C-terminal coding region of the cDNA before expression. The conditioned media from the transfected cells (0.5 to 2 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His-tagged constructs, the protein was purified using a Ni<sup>2+</sup>-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media was pumped onto a 6-ml Ni<sup>2+</sup>-NTA column equilibrated in 20 mM HEPES, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min at 4°C. After loading, the column was washed with additional equilibration buffer and the protein was eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM HEPES, 0.14 M NaCl, and 4% mannitol, pH 6.8, with a 25 ml G25 SUPERFINE<sup>TM</sup> (Pharmacia) column and stored at -80°C.

Immunoaderhin (Fc-containing) constructs of WISP-1 protein were purified from the conditioned media as follows. The conditioned media was pumped onto a 5-ml Protein A column (Pharmacia) which had been equilibrated in a 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1-ml fractions into tubes containing 275  $\mu$ l of 1 M Tris, pH 9, buffer. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His-

tagged proteins. The homogeneity of the protein was assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

#### EXAMPLE 11: Axis Duplication Assay

*Xenopus* embryos were injected with human WISP-2 mRNA into either a presumptive ventral or presumptive dorsal vegetal blastomere at the 8- to 16-cell stage to overexpress locally the encoded protein and assay for its developmental effects. The methods used are described in Sokol *et al.*, Cell, **67**: 741-752 (1991).

More specifically, for synthesis of capped RNA, human WISP-2 and mouse Wnt-1 cDNAs were cloned into the pGEMHE vector (gift of Dr. Todd Evans, AECOM) to prepare pGEMHE.hu.WISP-2.8H and pGEMHE.mu.Wnt-1, respectively. The constructs were linearized at the 3' end using the *SphI* restriction enzyme. Capped RNAs were synthesized using AMBION's T7 MESSAGEMACHINE™ RNA synthesis kit.

For obtaining mature oocytes, an adult female *Xenopus laevis* was injected with 200 I.U. pregnant mare serum 3 days before use. The night before the experiment, the female frog was injected with 800 I.U. of human chorionic gonadotropin. Fresh oocytes were squeezed from female frogs the next morning. *In vitro* fertilization of oocytes was performed by mixing oocytes with minced testes from a sacrificed male frog. Fertilized eggs were dejellied with 2% cysteine (pH 7.8) for 10 minutes. Dejellied eggs were washed once with distilled water and transferred to 0.1 x Modified Barth's Solution (MBS) (Methods in Cell Biology, Volume 36, *Xenopus laevis*: Practical uses in Cell and Molecular Biology, Kay and Peng, Eds (New York: Academic Press, 1991)) with 5% Ficoll. Eggs were lined on injection trays which contained 0.1 x MBS with 5% Ficoll for injection. After injection, embryos were kept in 0.1X MBS in an 18°C incubator. Embryos were staged according to Nieuwkoop and Faber, Normal Table of *Xenopus laevis*: (Daudin) (Amsterdam: North-Holland, 1967).

For animal cap assays, embryos were injected at the 2-cell stage with 1 ng of capped RNA, and animal caps were isolated at stage 8 and cultured in 1 x MMR for another 24 hours for the RT-PCR assay. Total RNA was isolated from harvested animal caps using a RNEASY™ kit (Qiagen). RNA samples (approximately 1 µg) were reverse transcribed using random hexamer and GIBCO BRL SUPERScript II™ reverse transcriptase. The annealing temperature for the PCR reactions was 55°C unless noted otherwise.

For axis duplication assays, embryos at the 8-cell stage were injected with 1 ng capped RNA at either the dorsal or ventral vegetal blastomere and incubated in 0.1X MBS for 72 hours.

The sequences of PCR primers used in this experiment were:

EF-1a.U:	5'-CAGATTGGTGGCTGGATATGC (SEQ ID NO:128)
EF-1a.D:	5'-ACTGCCTTGATTACTCCTAC (SEQ ID NO:129)
noggin.U:	5'-AGTTGCAGATGTGGCTCT (SEQ ID NO:130)
noggin.D:	5'-AGTCCAAGAGTCTCAGCA (SEQ ID NO:131)
goosecoid.U:	5'-ACAAGTGAAGCACTGGA (SEQ ID NO:132)
goosecoid.D:	5'-TCTTATTCCAGAGGAACC (SEQ ID NO:133)
cardiac-actin.U:	5'-TCCCTGTACGCTTCTGGTCGTA (SEQ ID NO:134)
cardiac-actin.D:	5'-TCTCAAAGTCCAAAGCCACATA (SEQ ID NO:135)

NCAM.U: 5'-CACAGTTCCAGCAAATAC (SEQ ID NO:136)

NCAM.D: 5'-GGAATCAGGCGGTACAGT (SEQ ID NO:137)

It was found that human WISP-2 can partially induce axis duplication in this assay.

**EXAMPLE 12: Thymidine Incorporation Assay**

5 In a ( $^3\text{H}$ )-thymidine incorporation assay, 19 different cell lines, including RAG (renal adenocarcinoma, mouse) and NRK-49F (normal kidney fibroblasts, rat) cells, identified in Table I below, were plated in 96-well plates at  $3 \times 10^4$  in HGDMEM with 10% serum. Twenty four hours after plating, the medium was changed to HGDMEM with 0.2% serum before adding the test proteins. WISP proteins were added to a final concentration of approximately 3.6 ng/ $\mu\text{l}$ . Serial dilutions were made in a total volume of 70  $\mu\text{l}$ /well of fresh media. After 18 hr incubation at 37°C, 5  $\mu\text{Ci/ml}$  ( $^3\text{H}$ )thymidine was added for 5 hrs. Medium was aspirated and cells were removed with 1X trypsin onto a GF/C filter using Packard's<sup>TM</sup> 96-well FILTERMATE 196<sup>TM</sup>. The filters were dried and 40  $\mu\text{l}$  of scintillation fluid was added for counting on a top count, microplate scintillation counter (Packard).

The results are shown in Table I:

TABLE I

$^3\text{H}$ -Thymidine Incorporation Assay Results

Cell line	Type	ATCC No.	mWISP-1 - IgG	hWISP-1 - IgG	hWISP-2 -IgG
HT-29 (human colon)	adenocarcinoma moderately well-differentiated	HTB-38	No change	No change	
Wi-Dr (human colon)	adenocarcinoma	CCL-218	No change	No change	
Calu-1 (human lung)	epidermoid carcinoma grade III, metastasis to pleura	HTB-54	inhibits ~1.1X	inhibits ~1.2X	
Calu-6 (human lung)	anaplastic carcinoma, probably lung	HTB-56	No change	stimulates ~1.4X	
SK-MES-1 (human lung)	squamous carcinoma, pleural effusion	HTB-58	No change	No change	
A549 (human lung)	carcinoma	CCL-185	inhibits ~1.5X	inhibits ~1.7X	
H460 (human lung)	large cell carcinoma	HTB-177	inhibits ~1.4X	inhibits ~1.3X	
SW900 (human lung)	squamous cell carcinoma	HTB-59	no change	no change	
MRC5 (human lung)	normal diploid	CCL-171	no change	no change	

	IMR-90 (human lung)	normal diploid	CCL-186	stimulates ~1.1X	stimulates ~1.5X	
5	Wnt-1 C57mg (mouse mammary gland)	myo-epithelial		inhibits ~2X		
	MLg (mouse lung)	lung		stimulates ~4X		
10	LL/2 (mouse lung)	lung carcinoma			inhibits ~2X	
	JC (mouse mammary gland)	carcinoma		inhibits ~2X	inhibits ~3X	
15	N MuMG (mouse mammary gland)	normal		stimulates ~2X	stimulates ~1.4X	
20	NRK-49F (rat kidney)	normal fibroblast		stimulates ~3X	stimulates ~3.5X	
	RAG (mouse kidney)	adenocarcinoma		stimulates ~4.5X	stimulates ~3X	stimulates ~4X
25	NIH/3T3 (mouse embryo)	fibroblast		stimulates ~3X		
	UCLA-P3 (human lung)	carcinoma		inhibits ~1.5X	inhibits ~2X	

It is seen that WISP-1 and WISP-2 exhibit both stimulatory and inhibitory effects on proliferation of normal and tumor cells, depending on the cell line employed.

### 30 EXAMPLE 13: Preparation of Antibodies that Bind WISP Polypeptide

#### 1. Polyclonal Antibodies

35 Polyclonal antisera were generated in female New Zealand White rabbits against murine WISP-1 and human WISP-2. The antigens used were proteins fused with histidine for murine WISP-1 and proteins fused with the Fc portion of IgG for human WISP-2. The same protocol was used for both proteins. Each protein was homogenized with Freund's complete adjuvant for the primary injection and with Freund's incomplete adjuvant for all subsequent boosts. For the primary immunization and the first boost, 3.3 µg per kg body weight was injected directly into the popliteal lymph nodes as described in Bennett *et al.*, J. Biol. Chem., 266: 23060-23067 (1991) and "Production of Antibodies by Inoculation into Lymph Nodes" by Morton Sigel *et al.* in Methods in Enzymology, Vol. 93 (New York: Academic Press, 1983). For all  
40 subsequent boosts, 3.3 µg per kg body weight was injected into subcutaneous and intramuscular sites. Injections were done every 3 weeks with bleeds taken on the following two weeks.

#### 2. Monoclonal Antibodies

Techniques for producing monoclonal antibodies that can specifically bind a WISP polypeptide are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified WISP polypeptide, fusion proteins containing WISP polypeptide, and cells expressing recombinant WISP polypeptide on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the WISP immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1 to 100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect antibodies to WISP polypeptide.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of a WISP polypeptide. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% PEG) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597, or x63.Ag8.653 (Kearney *et al.*, J. Immunology, 123: 1548 (1979)). The fusions generate hybridoma cells which can then be plated in 96-well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against a WISP polypeptide. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against a WISP polypeptide is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-WISP polypeptide monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel-exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

Specifically, for each of the human WISP-1 antibodies, five female Balb-c mice were pre-bled and then injected via their hind foot pads with purified human WISP-1, tagged with the Fc portion of IgG and emulsified prior to injection in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) in a 1:1 ratio of WISP antigen to adjuvant. The dosing schedule for the WISP-1 immunogen was as follows:

<u>Injection</u> <u>Date</u>	<u>Dose/Site</u>	<u>Dose/Animal</u>	<u>Concentration</u>
Day 16 of month 1	50 µl/site	100 µl/animal	6 µg/animal
Day 12 of month 2	50 µl/site	100 µl/animal	6 µg/animal
Day 21	50 µl/site	100 µl/animal	6 µg/animal

	of month 2			
	Day 28 of month 2	50 µl/site	100 µl/animal	2 µg/animal
5	Day 4 of month 3	50 µl/site	100 µl/animal	2 µg/animal
	Day 11 of month 3	50 µl/site	100 µl/animal	2 µg/animal
	Day 18 of month 3	50 µl/site	100 µl/animal	2 µg/animal
10	Day 25 of month 3	50 µl/site	100 µl/animal	2 µg/animal

For WISP-1, the mice were bled on Day 10 of month 4. After the mice were bled, the monoclonal antibodies were made by harvesting their spleens and by fusion as indicated above, using as the murine myeloma cell line X63.Ag8.653.

15 The five monoclonal antibodies generated to human WISP-1 are:

	10F2.2A7	gamma 2b/kappa
	10A9.2B1	gamma 2a/kappa
	8F7.1B1	gamma 1/kappa
	1H1.1D5	gamma 1/kappa
20	2G7.2H4	gamma 1/kappa

For WISP-2 monoclonal antibodies the same regimen is employed except that purified human WISP-2 is used as immunogen in the above protocol rather than purified human WISP-1 and the dosing schedule for the WISP-2 immunogen is as follows:

	<u>Injection</u> <u>Date</u>	<u>Dose/Site</u>	<u>Dose/Animal</u>	<u>Concentration</u>
25	Day 16 of month 1	50 µl/site	100 µl/animal	6 µg/animal
	Day 21 of month 2	50 µl/site	100 µl/animal	1 µg/animal
30	Day 28 of month 2	50 µl/site	100 µl/animal	1 µg/animal
	Day 4 of month 3	50 µl/site	100 µl/animal	1 µg/animal
35	Day 11 of month 3	50 µl/site	100 µl/animal	1 µg/animal
	Day 18 of month 3	50 µl/site	100 µl/animal	1 µg/animal
	Day 25 of month 3	50 µl/site	100 µl/animal	1 µg/animal

EXAMPLE 14: Uses of Antibodies that Bind WISP Polypeptide1. Cell lines

The established human breast tumor cells BT474 and MDA-MB-231 (which are available from ATCC) are grown in minimum essential medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT), sodium pyruvate, L-glutamine (2mM), non-essential amino acids, and 2x vitamin solution and maintained at 37°C in 5% CO<sub>2</sub>. Zhang *et al.*, Invas. & Metas., 11:204-215 (1991); Price *et al.*, Cancer Res., 50:717-721 (1990).

2. Antibodies

Anti-WISP-1 or anti-WISP-2 monoclonal antibodies that may be prepared as described above are harvested with PBS containing 25mM EDTA and used to immunize BALB/c mice. The mice are given injections i.p. of 10<sup>7</sup> cells in 0.5 ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated <sup>32</sup>P-labeled Wnt-1 are given i.p. injections of a wheatgerm agglutinin-SEPHAROSE<sup>TM</sup> (WGA)-purified Wnt membrane extract on weeks 9 and 13. This is followed by an i.v. injection of 0.1 ml of the Wnt-1 preparation, and the splenocytes are fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants are screened for Wnt-1 binding by ELISA and radioimmunoprecipitation. MOPC-21 (IgG1) (Cappell, Durham, NC) is used as an isotype-matched control.

Additionally, the anti-ErbB2 IgG<sub>1</sub>κ murine monoclonal antibodies 4D5 (ATCC CRL 10463 deposited May 24, 1990) and 7C2, specific for the extracellular domain of ErbB2, may be used with the above antibodies. They are produced as described in Fendly *et al.*, Cancer Research, 50:1550-1558 (1990) and WO89/06692.

3. Analysis of cell cycle status and viability

Cells are simultaneously examined for viability and cell cycle status by flow cytometry on a FACSTAR PLUS<sup>TM</sup> (Becton Dickinson Immunocytometry Systems USA, San Jose, CA). Breast tumor cells are harvested by washing the monolayer with PBS, incubating cells in 0.05% trypsin and 0.53 mM EDTA (Gibco), and resuspending them in culture medium. The cells are washed twice with PBS containing 1% FBS and the pellet is incubated for 30 minutes on ice with 50 µl of 400 µM 7-aminoactinomycin D (7AAD) (Molecular Probes, Eugene, OR), a vital dye which stains all permeable cells. Cells are then fixed with 1.0 ml of 0.5% paraformaldehyde in PBS and simultaneously permeabilized and stained for 16 hours at 4°C with 220 µl of 10 µg/ml HOECHST 33342<sup>TM</sup> dye (also a DNA binding dye) containing 5% TWEEN 20<sup>TM</sup>.

The data from 1 x 10<sup>4</sup> cells are collected and stored using LYSYS II<sup>TM</sup> software and analyzed using PAINT-A-GATE<sup>TM</sup> software (Becton Dickinson). Darzynkiewica *et al.*, Cytometry, 13:795-808 (1992); Picker *et al.*, J. Immunol., 150:1105-1121 (1993). The viability and percentage of cells in each stage of the cell cycle are determined on gated single cells using 7AAD and Hoechst staining, respectively. (Cell doublets are excluded by pulse analysis of width vs. area of the Hoechst signal.) Cell numbers are determined using a hemocytometer.

4. DNA synthesis (<sup>3</sup>H)-Thymidine Incorporation Assay

The assay was performed exactly as described in Example 12, except that the WISP polypeptides used as test proteins were replaced by the polyclonal antibodies generated in New Zealand White rabbits

against murine WISP-1 and human WISP-2 described in Example 13, and not all the cell lines in Example 12 were tested. The results are shown in Table II:

TABLE II  
<sup>3</sup>H-Thymidine Incorporation Assay Results

Cell line	Type	ATCC No.	pAB.mWISP-1	pAB.hWISP-2
HT-29 (human colon)	adenocarcinoma moderately well- differentiated	HTB-38	No change	No change
Wi-Dr (human colon)	adenocarcinoma	CCL-218	No change	No change
N MuMG (mouse mammary gland)	normal		inhibits ~3X	
NRK-49F (rat kidney)	normal fibroblast		stimulates ~2X	
RAG (mouse kidney)	adenocarcinoma		stimulates ~4X	
NIH/3T3 (mouse embryo)	fibroblast		inhibits ~2X	

It is seen that the polyclonal antibodies to mouse WISP-1 and to human WISP-2 exhibited both stimulatory and inhibitory effects on proliferation of normal and tumor cells, depending on the cell line employed.

#### 5. Affinity of binding to putative receptor

Radioiodinated anti-WISP-1 and anti-WISP-2 antibodies are prepared by the IODOGEN<sup>TM</sup> method. Fracker *et al.*, Biochem. Biophys. Res. Comm., 80:849-857 (1978). Binding assays are performed using appropriate receptor-expressing cells (such as, for mouse anti-WISP antibodies, MLG, a mouse lung cell line available from the ATCC) cultured in 96-well tissue culture plates (Falcon, Becton Dickinson Labware, Lincoln Park, N.J.). The cells are trypsinized and seeded in wells of 96-well plates at a density of 10<sup>4</sup> cells/well and allowed to adhere overnight. The monolayers are washed with cold culture medium supplemented with 0.1% sodium azide and then incubated in triplicate with 100 µl of serial dilutions of <sup>125</sup>I-anti-WISP-1 or WISP-2 antibodies in cold culture medium containing 0.1% sodium azide for 4 hours on ice. Non-specific binding is estimated by the preincubation of each sample with a 100-fold molar excess of nonradioactive antibodies in a total volume of 100 µl. Unbound radioactivity is removed by two washes with cold medium containing 0.1% sodium azide. The cell-associated radioactivity is detected in a gamma counter after solubilization of the cells with 150 µl of 0.1 M NaOH/well. The WISP-1 and WISP-2 binding constants (K<sub>d</sub>) and anti-WISP antibody binding affinities are determined by Scatchard analysis.

It is expected that the antibodies against WISP-1 and WISP-2 will affect the growth of these cells.

#### EXAMPLE 15: Further Uses of Antibodies that Bind WISP Polypeptide

##### 1. WISP-1 and WISP-2

This example shows that the WISP-1 and WISP-2 genes are amplified in the genome of certain human lung, colon, and/or breast malignant tumors and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the WISP-1 and WISP-2 proteins are useful targets for



therapeutic intervention in certain cancers such as colon, lung, breast, and other cancers. A therapeutic agent may take the form of antagonists of WISP molecules, for example, murine-human, chimeric, humanized, or human antibodies against WISP-1 and WISP-2, such as the antibodies prepared as described above.

The starting material for the screen was genomic DNA isolated from a variety of cancers. The DNA is quantitated precisely, e.g., fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals, pooled, and used as an assay control for the gene copy in healthy individuals.

The 5' nuclease assay (for example, TAQMAN<sup>TM</sup>) and real-time quantitative PCR (for example, ABI PRIZM 7700<sup>TM</sup> Sequence Detection System<sup>TM</sup> (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNAs encoding WISP-1 and WISP-2 are over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table III. An explanation of the abbreviations used for the designation of the primary tumors listed in Table III and the primary tumors and cell lines referred to throughout this example is given below:

Human lung carcinoma cell lines include A549 (SRCC768), Calu-1 (SRCC769), Calu-6 (SRCC770), H157 (SRCC771), H441 (SRCC772), H460 (SRCC773), SKMES-1 (SRCC774) and SW900 (SRCC775), all available from ATCC. Primary human lung tumor cells usually derive from adenocarcinomas, squamous cell carcinomas, large cell carcinomas, non-small cell carcinomas, small cell carcinomas, and broncho alveolar carcinomas, and include, for example, SRCC724 (squamous cell carcinoma abbreviated as "SqCCa"), SRCC725 (non-small cell carcinoma, abbreviated as "NSCCa"), SRCC726 (adenocarcinoma, abbreviated as "AdenoCa"), SRCC727 (adenocarcinoma), SRCC728 (squamous cell carcinoma), SRCC729 (adenocarcinoma), SRCC730 (adeno/squamous cell carcinoma), SRCC731 (adenocarcinoma), SRCC732 (squamous cell carcinoma), SRCC733 (adenocarcinoma), SRCC734 (adenocarcinoma), SRCC735 (broncho alveolar carcinoma, abbreviated as "BAC"), SRCC736 (squamous cell carcinoma), SRCC738 (squamous cell carcinoma), SRCC739 (squamous cell carcinoma), SRCC740 (squamous cell carcinoma), and SRCC740 (lung cell carcinoma, abbreviated as "LCCa").

Colon cancer cell lines include, for example, ATCC cell lines SW480 (adenocarcinoma, SRCC776), SW620 (lymph node metastasis of colon adenocarcinoma, SRCC777), COLO320 (adenocarcinoma, SRCC778), HT29 (adenocarcinoma, SRCC779), HM7 (carcinoma, SRCC780), CaWiDr (adenocarcinoma, srcc781), HCT116 (carcinoma, SRCC782), SKCO1 (adenocarcinoma, SRCC783), SW403 (adenocarcinoma, SRCC784), LS174T (carcinoma, SRCC785), and HM7 (a high mucin producing variant of ATCC colon adenocarcinoma cell line LS 174T, obtained from Dr. Robert Warren, UCSF). Primary colon tumors include colon adenocarcinomas designated CT2 (SRCC742), CT3 (SRCC743), CT8 (SRCC744), CT10 (SRCC745), CT12 (SRCC746), CT14 (SRCC747), CT15 (SRCC748), CT17 (SRCC750), CT1 (SRCC751), CT4 (SRCC752), CT5 (SRCC753), CT6 (SRCC754), CT7 (SRCC755), CT9 (SRCC756), CT11 (SRCC757), CT18 (SRCC758), and DcR3, BACrev, BACfwd, T160, and T159.

Human breast carcinoma cell lines include, for example, HBL100 (SRCC759), MB435s (SRCC760), T47D (SRCC761), MB468 (SRCC762), MB175 (SRCC763), MB361 (SRCC764), BT20 (SRCC765), MCF7 (SRCC766), and SKBR3 (SRCC767).

The results are reported in delta ( $\Delta$ ) CT units. One unit corresponds to one PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers derived from the 3'-untranslated regions of the *WISP-1* and *WISP-2* cDNAs and a TAQMAN<sup>TM</sup> fluorescent probe corresponding to the respective intervening sequences. Using the 3' region tends to avoid crossing intron-exon boundaries in the genomic DNA, an essential requirement for accurate assessment of gene amplification using this method. The sequences for the primers and probes (forward, reverse, and probe) used for the *WISP-1*-encoding and *WISP-2*-encoding gene amplification were as follows:

WISP-1 probe and primers:

10 hu.WISP1.TMP (probe) 5'-AGCCTTTCCAAGTCACTAGAAGTCCTGCTGG (SEQ ID NO:138)

hu.WISP1.TMF (forward primer) 5'-CTGGACTACACCCAAGCCTGA (SEQ ID NO:139)

hu.WISP1.TMR (reverse primer) 5'-CATTTCTTGGGATTTAGGCAAGA (SEQ ID NO:140)

WISP-2 probe and primers:

DNA33473.3utr-5 (forward primer) 5'-TCTAGCCCCACTCCCTGCCT (SEQ ID NO:141)

15 DNA33473.3utr-3 (reverse primer) 5'-GAAGTCGGAGAGAAAGCTCGC (SEQ ID NO:142)

DNA33473.3utr-probe 5'-CACACACAGCCTATATCAAACATGCACACG (SEQ ID NO:143)

The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRISM 7700<sup>TM</sup> Sequence Detection System<sup>TM</sup>. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

35 5'-Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The  $\Delta$ Ct values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

The results of the first run performed are shown in Figures 19A-D and 20A-D for *WISP-1* and *WISP-2*, respectively, and controls. Note the pattern shown in Fig. 19B (marked huWISP-1). The standard

deviation for two samples of normal human DNA is shown in the column marked Nor Hu. This was used as a quality control tool. If the standard deviation was unacceptably large, the entire run was repeated. The nine additional columns corresponded to the human colon cancer cell lines noted above. The delta CT's for HT29 and WIDr were >3, corresponding to an about 8-fold over-representation of the *WISP-1* gene in these samples compared to the normal samples. Similarly, Fig. 19B suggests an about 4-fold amplification of *WISP-1* in the HCT116, SKCo-1, and SW403 cell lines.

As a comparison, see Fig. 20B (marked huFASr). The generally small delta CT values indicate that this gene was not significantly amplified in any of the cell lines (the value of 1 for SW620 corresponding to 2-fold amplification is within the noise level for the assay).

The *WISP-1* result was confirmed in three replicate reactions. See Figures 21A-D, 22A-D, and 23A-C. The pattern and delta CT values obtained were very similar in Figures 21A-C (marked huWISP-1c, huWISP-1b, and huWISP-1a, respectively). The result was essentially identical to that obtained in the first run. HT29 and WIDr showed the highest levels of *WISP-1* amplification, while HCT116, SKCo-1, and SW403 cell lines showed somewhat lower levels of *WISP-1* gene amplification. Two additional reactions from a third run were confirmatory. See Figs. 25A and 25B.

The *WISP-1* gene is located on chromosome 8, in the general vicinity of the *myc* gene, which is known to be amplified in some colon cancer cell lines. The pattern obtained using primers and probe for the *myc* gene, namely,

hu.c-myc.tm.p 5'-CTTGAG<sup>3</sup> CTGAAAGATTTAGCCATAATGTAAACTGCCT (SEQ ID NO:144)

hu.c-myc.tm.f 5'-CAAATGCAACCTCACAACCTTG (SEQ ID NO:145), and

hu.c-myc.tm.r 5'-TTCTTTTATGCCCCAAGTCCAATT (SEQ ID NO:146).

is consistent with a published report (Cancer Research, 57: 1769-1775 (1997)), tending to validate the 5' nuclease assay method, but is clearly different from that obtained for *WISP-1*. These data prove that the *myc* gene is not the target of the amplification detected using the primers and probes for *WISP-1*.

The data using primers and probes based on the *WISP-2* DNA sequence suggest that this gene may be the target of low-level gene amplification in most of the cell lines examined. See Figs. 20C, 22A-D, and 25C and D. Hence, antibodies to both *WISP-1* and *WISP-2*, particularly humanized antibodies, are expected to be of benefit in combating certain types of cancer such as colon cancer, similar to the humanized anti-HER-2 antibody in clinical use.

## 2. *WISP-2*

### Description of Tumors and Cell Lines

Amplification using several different tumor types was performed for human *WISP-2* (PRO261), as described below. Table III describes the stage, T stage, and N stage of various primary tumors which were used to screen the *WISP-2* compound of the invention.

TABLE III  
Primary Lung and Colon Tumor Profiles

	Primary Tumor	Stage	Other Stage	Dukes Stage	T Stage	N Stage
	Human lung tumor SqCCA (SRCC724) [LT1]	IB	--	--	T1	N1
5	Human lung tumor NSCCa (SRCC725) [LT1a]	IA	--	--	T3	NO
	Human lung tumor AdenoCa (SRCC726) [LT2]	IB	--	--	T2	NO
	Human lung tumor AdenoCa (SRCC727) [LT3]	IB	--	--	T1	N2
	Human lung tumor SqCCq (SRCC728) [LT4]	IIB	--	--	T2	NO
	Human lung tumor AdenoCa (SRCC729) [LT6]	IV	--	--	T1	NO
10	Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IB	--	--	T1	NO
	Human lung tumor AdenoCa (SRCC731) [LT9]	IIB	--	--	T2	NO
	Human lung tumor SqCCa (SRCC732) [LT10]	IA	--	--	T2	N1
	Human lung tumor AdenoCa (SRCC733) [LT11]	IB	--	--	T1	N1
	Human lung tumor AdenoCa (SRCC734) [LT12]	IIA	--	--	T2	NO
15	Human lung tumor BAC (SRCC735) [LT13]	IB	--	--	T2	NO
	Human lung tumor SqCCa (SRCC736) [LT15]	IB	--	--	T2	NO
	Human lung tumor SqCCa (SRCC737) [LT16]	IB	--	--	T2	NO
	Human lung tumor SqCCa (SRCC738) [LT17]	IIB	--	--	T2	N1
	Human lung tumor SqCCa (SRCC739) [LT18]	IB	--	--	T2	NO
20	Human lung tumor SqCCa (SRCC740) [LT19]	IB	--	--	T2	NO
	Human lung tumor LCCa (SRCC741) [LT21]	IIB	--	--	T3	N1
	Human colon AdenoCa (SRCC742) [CT2]	--	M1	D	pT4	NO
	Human colon AdenoCa (SRCC743) [CT3]		--	B	pT3	NO
	Human colon AdenoCa (SRCC 744) [CT8]			B	T3	NO
25	Human colon AdenoCa (SRCC745) [CT10]			A	pT2	NO
	Human colon AdenoCa (SRCC746) [CT12]		MO, R1	B	T3	NO

	Human colon AdenoCa (SRCC747) [CT14]		pMO, RO	B	pT3	pNO
	Human colon AdenoCa (SRCC748) [CT15]		M1, R2	D	T4	N2
	Human colon AdenoCa (SRCC749) [CT16]		pMO	B	pT3	pNO
	Human colon AdenoCa (SRCC750) [CT17]			C1	pT3	pN1
5	Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	NO
	Human colon AdenoCa (SRCC752) [CT4]			B	pT3	MO
	Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pNO
	Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pNO
	Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pNO
10	Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
	Human colon AdenoCa (SRCC757) [CT11]			B	T3	NO
	Human colon AdenoCa (SRCC758) [CT18]		MO, RO	B	pT3	pNO

#### DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, and normal human blood (controls and framework and epicenter mapping). The isolation was performed using purification kit #13362 (which includes 10 purification tips with a capacity of 400 µg genomic DNA each), buffer set #1960 and protease #19155 and #19101, all from Quiagen, according to the manufacturer's instructions and the description below.

#### Cell culture lysis:

Cells were washed and trypsinized at a concentration of  $7.5 \times 10^8$  per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS recentrifugation. The pellets were washed a third time, and the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 mL PBS. Buffer C1 was equilibrated at 4°C. Protease #19155 (Quiagen) was diluted into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 mL of G2 Buffer was prepared by diluting RNase A stock (Quiagen) (100 mg/ml) to a final concentration of 200 µg/ml.

Buffer C1 (10 mL, 4°C) and ddH<sub>2</sub>O (40 mL, 4°C) were then added to the 10 mL of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a BECKMAN™ swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the nuclei were suspended with a vortex into 2 mL Buffer C1 (at 4°C) and 6 mL ddH<sub>2</sub>O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200 µl per tip. G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Quiagen protease (200 µl, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (e.g., incubating an additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Solid human tumor sample preparation and lysis:*

Tumor samples were weighed and placed into 50-ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNase A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenized in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar-flow TC hood to order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH<sub>2</sub>O, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Protease (Quiagen), prepared as indicated above, 1.0 ml, was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation were repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Human blood preparation and lysis:*

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Protease (Quiagen) was freshly prepared by dilution into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNase A to a final concentration of 200 µg/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50-ml conical tube, and 10 ml C1 buffer and 30 ml ddH<sub>2</sub>O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a BECKMAN<sup>TM</sup> swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant was discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH<sub>2</sub>O (4°C). Vortexing was repeated until the pellet was white. The nuclei were then suspended into the residual buffer using a 200-µl tip. G2 buffer (10 ml) was added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Protease (Quiagen) was added (200 µl) and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (e.g., incubating an additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Purification of cleared lysates: Isolation of genomic DNA:*

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30-ml silanized, autoclaved 30-ml COREX<sup>TM</sup> tubes with 15-ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, and the tubes were covered with paraffin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5-

ml tubes with a 26-gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

*Quantitation of genomic DNA and preparation for gene amplification assay:*

The DNA levels in each tube were quantified by standard A260, A280 spectrophotometry on a 1:20 dilution (5 µl DNA + 95 µl ddH<sub>2</sub>O) using the 0.1-ml quartz cuvettes in the BECKMAN DU640<sup>TM</sup> spectrophotometer. A260/A280 ratios were in the range of 1.8-1.9. Each DNA sample was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/µl), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a HOFFER DYNA QUANT 200<sup>TM</sup> fluorometer to warm up for about 15 minutes. The HOECHST<sup>TM</sup> dye working solution (#H33258, 10 µl, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2-ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2 µl, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. A second 2 µl of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometrically-determined concentration was then used to dilute each sample to 10 ng/µl in ddH<sub>2</sub>O. This was done simultaneously on all template samples for a single TAQMAN<sup>TM</sup> plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used, provided that the CT value of normal human DNA subtracted from test DNA was +/- 1 CT. The diluted, lot-qualified genomic DNA was stored in 1.0-ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1-ml aliquot is enough for 8-9 plates or 64 tests.

Framework Mapping and Epicenter Marking:

Human WISP-I was reexamined with both framework and epicenter mapping. Selected tumors from the above initial screen were reexamined with both framework and epicenter mapping. Table IV indicates the chromosomal mapping of the framework markers that were used in the present example. The framework markers are located approximately every 20 megabases along Chromosome 8 and were used to control for aneuploidy.

TABLE IV  
Framework Markers

Map Position on Chromosome 8	Stanford Human Genome Center Marker Name
H9	EST-00040
H59	WI-961
H121	SHGC-11323
H200	SHGC-7433
H256	AFMa183zf1

Table V describes the epicenter markers that were employed in association with WISP-1. These markers are located in close proximity to the gene for WISP-1 and are used to assess the amplification status of the region of chromosome 8 in which the gene for WISP-1 is located. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-32958 is the marker found to be the closest to the location on chromosome 8 to which the gene encoding WISP-1 most closely maps.

TABLE V  
Epicenter Markers

Map Position on Chromosome 8	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
H257	AFMa248tel	103(gap)
H259	SHGC-36664	33
H261	AFM259xc5	63
H266	SHGC-32958	41
H267	AFMa175xc1	19
H268	AFM337wg5	87
H273	SHGC-33759	71
H274	SHGC-32752	5
H275	WI-7711	21
H277	SHGC-34940	-

The framework markers for human WISP-2 are located approximately every 20 megabases along Chromosome 20, and are used to control for aneuploidy. The markers are shown in Table VI.

TABLE VI  
Framework Markers

Map Position on Chromosome 20	Stanford Human Genome Center Marker Name
T10	SHGC-2797
T48	UT759
T73	AFMa339xf5
T115	SHGC-33922
T159	SHGC-36268

The marker SHGC-33922 is the marker to which human WISP-2 DNA most closely maps. This marker is between the framework markers. Framework analysis showed that all markers were up in tumors; thus, chromosome 20 was aneuploid in many tumors. Since the markers were up due to aneuploidy, epicenter analysis was not done for human WISP-2 gene.



The  $\Delta C_t$  values of the above described framework markers along Chromosome 8 relative for WISP-1 are indicated for selected tumors in Tables VII and VIII.

TABLE VII

Amplification of framework markers relative to Human WISP-1 DNA Framework Markers ( $\Delta$ ct)

		Probe/Delta CT							
	Template	c-myc (SD)	WISP-1 (SD)	WISP-2 (SD)	H9 (SD)	H59 (SD)	H121 (SD)	H200 (SD)	H256 (SD)
	Nor Hu	0.00 (0.91)	0.00 (0.01)	0.00 (0.20)	0.00 (0.13)	0.00 (0.20)	0.00 (0.14)	0.00 (0.16)	0.00 (0.04)
5	SW480	1.86	0.84	1.92	-1.18	1.01	0.17	0.65	0.81
	SW620	1.45	0.98	1.60	0.45	0.75	1.00	0.81	0.52
	Colo320	3.73	0.65	1.88	0.69	0.70	0.89	0.60	0.40
	HT29	0.83	2.67	2.20	-1.13	-0.40	-0.55	1.00	2.42
	HM7	-2.03	0.07	-0.28	-0.28	0.24	-0.48	0.12	-0.26
10	WiDr	-0.13	2.91	1.67	-0.20	0.95	0.07	1.43	2.55
	HCT116	-0.57	1.82	1.04	1.24	1.56	0.84	1.76	1.53
	SKCO-1	0.19	1.68	0.97	-0.30	0.32	0.12	1.39	1.63
	SW403	-0.72	1.34	1.77	0.23	0.53	0.26	1.48	1.48
15	Nor Hu	----	0.00 (0.18)	0.00 (1.02)	0.00 (0.08)	0.00 (0.13)	0.00 (0.01)	0.00 (0.16)	0.00 (0.37)
	CT-2	----	0.65	0.44	-0.25	0.11	0.07	0.13	0.95
	CT-3	----	0.90	0.95	-0.27	0.05	-0.10	-0.11	0.32
	CT-8	----	0.47	-0.34	0.07	-0.20	0.00	-0.04	0.07
	CT-10	----	0.76	0.50	0.23	-0.36	-0.08	0.17	0.70
20	CT-12	----	1.30	2.14	-0.70	-0.45	0.24	0.47	1.75
	CT-14	----	1.17	-0.48	0.05	0.18	0.31	0.23	1.51
	CT-15	----	0.22	-0.13	0.13	-0.48	0.29	0.11	0.59
	CT-16	----	0.26	0.10	0.00	-0.15	-0.23	-0.09	0.95
	CT-17	----	0.57	-0.33	0.73	-0.11	-0.05	-0.11	0.25
25									
	Nor Hu	----	0.00(0.45)	0.00 (1.07)	0.000 (0.04)	0.00 (0.21)	0.00 (0.18)	0.00 (0.03)	0.00 (0.18)
	CT-1	----	0.84	-0.37	-0.36	0.19	0.68	0.01	0.66
	CT-4	----	0.15	-0.23	-1.00	0.24	-0.11	0.30	0.14
	CT-5	----	0.86	-1.23	-0.60	-0.25	0.22	0.51	0.62

CT-6	----	0.03	0.39	-0.24	0.61	0.70	0.01	0.19
CT-7	----	-0.20	-1.36	-0.76	0.00	-0.09	-0.13	-0.18
CT-9	----	0.30	-0.54	-0.50	0.29	0.54	0.11	0.18
CT-11	----	0.48	0.14	-0.89	0.34	0.82	0.17	-0.06
CT-18	----	-0.20	-1.37	-0.52	0.32	0.66	0.08	0.12

TABLE VIII

Amplification of framework markers relative to Human WISP-1 DNA Framework Markers ( $\Delta$ Ct)

Template	Probe/Delta CT					
	WISP-2 (SD)	T10 (SD)	T48 (SD)	T73 (SD)	T115 (SD)	T159 (SD)
Nor Hu	0.00 (0.05)	0.00 (0.16)	0.00 (0.09)	0.00 (0.21)	0.00 (3.22)	0.00 (0.09)
SW480	1.31	1.32	0.63	1.94	-5.66	1.61
SW620	1.32	2.02	1.42	1.06	-10.95	1.48
Colo320	0.43	1.35	1.37	0.61	0.30	1.37
HT29	1.76	1.09	-2.23	1.26	-5.47	1.87
HM7	-0.32	0.32	0.38	0.41	-6.3	0.48
WiDr	1.76	1.61	-1.38	1.04	-7.36	1.55
HCT116	1.18	1.24	1.15	1.46	-8.38	1.49
SKCO-1	1.40	1.17	1.19	1.13	-5.34	1.61
SW403	1.92	2.24	-17.23	1.38	-3.66	2.12

Gene Amplification Assay Results:

The human WISP-2 (PRO261) compound of the invention was screened in the following primary tumors and the resulting  $\Delta$ Ct values are reported in Table IX.

TABLE IX

 $\Delta$ Ct values in lung and colon primary tumor models

Primary Tumor	PRO261
LT1	0.41
LT1a	1.08
LT2	0.27
LT3	0.98
LT4	0.32

	LT6	0.45
	LT7	0.03
	LT9	0.18
	LT10	1.16
5	LT11	0.67, 1.59, 0.63, 0.19.
	LT12	0.80, 1.73, 1.08, 2.23
	LT13	1.02, 1.13, 1.01, 0.29
	LT15	0.97, 2.64, 0.56, 2.38
	LT16	0.80, 0.75, 0.82, 2.05
10	LT17	1.67, 2.01, 1.43, 0.93
	LT18	1.22, 0.46, 0.15, -0.17
	LT19	0.78, 1.38, 1.39, 2.33
	LT21	0.04, 1.14, 0.48, 3.40
	CT2	1.66
15	CT3	2.14
	CT8	0.55
	CT10	1.00
	CT12	0.34
	CT14	1.03
20	CT15	0.67
	CT16	0.87
	CT17	-0.19
	CT1	-0.06
	CT4	1.00
25	CT5	1.07
	CT6	-0.08
	CT7	0.15
	CT9	0.68
	CT11	0.59
30	CT18	0.73
	A549	--
	Calu-1	--
	Calu-6	--

	H157	--
	H441	--
	H460	--
	SKMES1	--
5	SW900	--
	SW480	0.62, 1.90, 1.20, 1.57, 1.68, 1.36, 1.59, 1.86, 1.91, 2.36, 1.68, 1.53, 2.50
	SW620	0.66, 1.65, 1.85, 1.63, 1.61, 1.24, 1.52, 1.98, 1.57, 1.83, 1.41, 1.42, 1.59
	Colo320	-0.33, 0.66, 0.48, 0.91, 0.72, 0.33, 2.49, 0.99, 1.06, 1.24, 1.04, 0.46, 0.27
	HT29	0.46, 1.95, 1.61, 2.58, 1.49, 1.38, 1.40, 2.00, 2.59, 2.59, 1.39, 1.32
10	HM7	-0.70, 0.74, -0.29, 0.66, 0.27, 0.08, 0.54, 0.67, 0.64, 0.34, 0.09, 0.29, 0.21
	WiDr	0.19, 1.64, 1.00, 1.71, 1.44, 1.57, 0.93, 1.84, 1.58, 0.91, 0.87
	HCT116	0.25, 1.29, 1.04, 2.01, 1.29, 1.07, 1.08, 2.05, 1.81, 1.56, 1.05, 1.09, 0.96
	SKCO1	0.73, 1.99, 1.33, 1.00, 1.33, 1.26, 1.19, 2.10, 1.50, 2.13, 1.33, 1.29
	SW403	0.26, 1.98, 1.42, 2.20, 2.40, 1.50, 1.43, 2.15, 1.52, 1.67, 2.19, 1.40, 1.29
15	LS174T	1.48
	HBL100	1.40
	MB435s	1.43
	T47D	0.38
	MB468	-0.08
20	MB175	0.23
	MB361	0.37
	BT20	1.66
	MCF7	0.53
	SKBR3	1.73

25 The  $\Delta C_t$  values for DNA33473 (PRO261; human WISP-2) in a variety of primary lung and colon tumors as well as lung tumor cell lines are reported in Table IX. A  $\Delta C_t$  value of  $> 1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of the gene copy. Table IX indicates that significant amplification of DNA33474 occurred in: (1) primary lung tumors LT1a, LT10,

LT12, LT15, LT17 and LT19; (2) primary colon tumors CT2, CT3, CT14, and CT5; (3) colon tumor cell lines SW480, SW620, HT29, WiDr, HCT116, SKCO1, SW403, and LS174T and (4) breast tumor cell lines HBL100, MB435s, BT20 and SKBR3.

The  $\Delta C_t$  and average  $\Delta C_t$  values for the primary lung tumors were the following: 1.08, 1.16, 1.17, 1.64, 1.50 and 1.47, respectively; those for the primary colon tumors were 1.16, 2.14, 1.03 and 1.07, respectively; those for the colon tumor cell lines were 1.67, 1.54, 1.73, 1.24, 1.32, 1.35, 1.65, and 1.48, respectively; and those for the breast tumor cell lines were 1.40, 1.43, 1.66, and 1.73, respectively.

For the lung tumors, this represents approximately a 2.1-, 2.2-, 2.2-, 3.1-, 2.8-, and 2.8-, respectively, fold increase in gene copy relative to normal tissue. For the colon tumors, this represents a 2.2-, 4.4-, 2.0-, and 2.1-, respectively, fold increase in gene copy relative to normal tissue. For the colon tumor cell lines, this represents a 3.2-, 2.9-, 3.3-, 2.4-, 2.5-, 2.5-, 3.1-, and 2.8-, respectively, fold increase in gene copy relative to normal tissue. For the breast tumor cell lines, this represents a 2.6-, 2.7-, 3.2-, and 3.3-, respectively, fold increase in gene copy relative to normal tissue. Because amplification of DNA33473 (PRO261) occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists (e.g., antibodies) directed against the protein encoded by DNA33473 (PRO261) would be expected to be useful in cancer therapy.

#### EXAMPLE 16: *In Situ* Hybridization

*In situ* hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, in identifying sites of gene expression, analyzing the tissue distribution of transcription, identifying and localizing viral infection, following changes in specific mRNA synthesis, and aiding in chromosome mapping.

*In situ* hybridization was performed following an optimized version of the protocol by Lu and Gillett, Cell Vision 1: 169-176 (1994), using PCR-generated  $^{33}\text{P}$ -labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A ( $^{33}\text{P}$ -UTP)-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in KODAK NTB2<sup>TM</sup> nuclear track emulsion and exposed for 4 weeks.

#### $^{33}\text{P}$ -Riboprobe synthesis

6.0  $\mu\text{l}$  (125 mCi) of  $^{33}\text{P}$ -UTP (Amersham BF 1002, SA < 2000 Ci/mmol) were speed-vacuum dried. To each tube containing dried  $^{33}\text{P}$ -UTP, the following ingredients were added:

- 2.0  $\mu\text{l}$  5x transcription buffer
- 1.0  $\mu\text{l}$  DTT (100 mM)
- 2.0  $\mu\text{l}$  NTP mix (2.5 mM : 10  $\mu\text{l}$  each of 10 mM GTP, CTP & ATP + 10  $\mu\text{l}$  H<sub>2</sub>O)
- 1.0  $\mu\text{l}$  UTP (50  $\mu\text{M}$ )
- 1.0  $\mu\text{l}$  RNAsin
- 1.0  $\mu\text{l}$  DNA template (1  $\mu\text{g}$ )

1.0  $\mu$ l H<sub>2</sub>O

1.0  $\mu$ l RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

The tubes were incubated at 37°C for one hour. A total of 1.0  $\mu$ l RQ1 DNase was added, followed by incubation at 37°C for 15 minutes. A total of 90  $\mu$ l TE (10 mM Tris pH 7.6/1 mM EDTA, pH 8.0) was added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a  
5 MICROCON-50<sup>TM</sup> ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, a total of 100  $\mu$ l TE was added. Then 1  $\mu$ l of the final product was pipetted on DE81 paper and counted in 6 ml of BIOFLUOR II<sup>TM</sup>.

10 The probe was run on a TBE/urea gel. A total of 1-3  $\mu$ l of the probe or 5  $\mu$ l of RNA Mrk III was added to 3  $\mu$ l of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, and the sample was loaded and run at 180-250 volts for 45 minutes. The gel was wrapped in plastic wrap (SARAN<sup>TM</sup> brand) and exposed to XAR film with an intensifying screen in a -70°C freezer one hour to overnight.

15 <sup>33</sup>P-Hybridization

A. Pretreatment of frozen sections

The slides were removed from the freezer, placed on aluminum trays, and thawed at room temperature for 5 minutes. The trays were placed in a 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and  
20 washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975 ml s.c. H<sub>2</sub>O). After deproteinization in 0.5  $\mu$ g/ml proteinase K for 10 minutes at 37°C (12.5  $\mu$ l of 10 mg/ml stock in 250 ml prewarmed RNase-free RNase buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, and 100% ethanol, 2 minutes each.

B. Pretreatment of paraffin-embedded sections

25 The slides were deparaffinized, placed in s.c. H<sub>2</sub>O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were deproteinized in 20  $\mu$ g/ml proteinase K (500  $\mu$ l of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) for human embryo tissue, or 8 x proteinase K (100  $\mu$ l in 250 ml RNase buffer, 37°C, 30 minutes) for formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

30 C. Prehybridization

The slides were laid out in a plastic box lined with Box buffer (4 x SSC, 50% formamide) The filter paper was saturated. The tissue was covered with 50  $\mu$ l of hybridization buffer (3.75 g dextran sulfate + 6 ml s.c. H<sub>2</sub>O), vortexed, and heated in the microwave for 2 minutes with the cap loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC, and 9 ml s.c. H<sub>2</sub>O were added, and the tissue was vortexed  
35 well and incubated at 42°C for 1-4 hours.

D. Hybridization

1.0 x 10<sup>6</sup> cpm probe and 1.0 µl tRNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µl hybridization buffer was added per slide. After vortexing, 50 µl <sup>33</sup>P mix was added to 50 µl prehybridization on the slide. The slides were incubated overnight at 55°C.

5 E. Washes

Washing was done for 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25 M EDTA, V<sub>f</sub>=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µl of 10 mg/ml in 250 ml RNase buffer = 20 µg/ml). The slides were washed 2x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA  
10 (20 ml 20 x SSC + 16 ml EDTA, V<sub>f</sub>=4L).

F. Oligonucleotides

*In situ* analysis was performed on DNA sequences disclosed herein. The oligonucleotides employed for these analyses are as follows.

(1) Mouse WISP-1 (Clone 568)

15 Notrim-p1: 5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GTC CCT GGC CAG TGC TGT GAG-3'  
(SEQ ID NO:147)

Notrim-p2: 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GGG CCA GGC TTT GCT TCC ATT-3'  
(SEQ ID NO:148)

(2) Human WISP-1

20 hmWISP-1 p1: 5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC TGG AGG CAT GGC ACA GGA  
AC-3' (SEQ ID NO:149)

hmWISP-1 p2: 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA TCC GGA TCA GGC TTG GGT  
GTA-3' (SEQ ID NO:150)

(3) Mouse WISP-2 (Clone 1367.3)

25 1367.p1: 5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC AGC TTG GGA TGG AGG TCT TTC-3'  
(SEQ ID NO:151)

1367.p2: 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GGG CAC TGG GGT GGT GT-3' (SEQ  
ID NO:152)

(4) Human WISP-2 (DNA33473)

30 DNA33473-p1: 5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GCG AGG ACG GCG GCT TCA-3'  
(SEQ ID NO:153)

DNA33473-p2: 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA AGA GTC GCG GCC GCC CTT  
TTT-3' (SEQ ID NO:154)

(5) Human WISP-3

35 WISP3-p1: 5'-GGA TTC TAA TAC GAC TCA CTA TAG GGC GGG GCT CCT CTT CTC CAC TCT-3'  
(SEQ ID NO:155)



WISP3-p2 5'-CTA TGA AAT TAA CCC TCA CTA AAG GGA GCT GTC GCA AGG CTG AAT GTA-3'  
(SEQ ID NO:156)

#### G. Results

*In situ* analysis was performed on the above DNA sequences disclosed herein. The results from these analyses are as follows.

##### (1) Mouse WISP-1

##### Expression in Mouse Tissues

Mouse Fetal Tissues: *In situ* hybridization of mouse WISP-1 showed strong expression in embryonic mesenchymal tissues. At E10.5 expression was observed in tissues that would develop into skeletal elements in the adult; this pattern was maintained at later stages of embryonic development. In later stages (E12.5 and E15.5), expression was highest in osteoblasts at the sites of bone formation. Expression was also observed in the embryonic heart, where the signal was particularly strong in the atria at E12.5 (atria were not included in sections at E15.5).

Mouse Adult Tissues: No expression was observed in any of the adult tissues examined, including heart, lung, kidney, adrenal, liver, pancreas, cerebrum, and cerebellum. These results do not correlate with the Northern data.

Additional sites of expression in the fetus were the walls of developing blood vessels and in fibroblast-like cells within the hepatic portal tract mesenchyme.

##### Expression in Normal and Wnt-1 Transgenic Tumors

Expression with the antisense probe was observed over fibroblast-like cells lying adjacent to the subcutaneous skeletal muscle in P10 (post-natal day 10 pups) and in pregnant females. Expression was not observed over breast epithelial cells at any of the time points examined in the study.

Expression of mouse WISP-1 was high in all three of the Wnt-1 transgenic tumors tested and appeared to be confined to the supporting fibroblast-like cells within the delicate connective tissue stroma. Some expression was seen over the tumor cells themselves; however, this likely represents overspill from tumor fibroblasts, rather than true expression by tumor cells.

In summary, mouse WISP-1 was expressed in embryonic skeletal mesenchyme and at sites of bone formation. It was additionally expressed in fibroblasts in the sub-cutis of growing pups and pregnant females. It is likely to play a role in osteogenesis, and may be involved in repair after injury. Expression was also observed in the embryonic heart.

##### (2) Human WISP-1

##### Expression in Human Tissues

Human Fetal Tissue The fetal tissues examined (E12-E16 weeks) included: placenta, umbilical cord, liver, kidney, adrenals, thyroid, lungs, heart, great vessels, oesophagus, stomach, small intestine, spleen, thymus, pancreas, brain, eye, spinal cord, body wall, pelvis, and lower limb.

Human WISP-1 exhibited expression at sites of connective tissue interfaces in the fetus, for example, developing portal tracts, fascial planes in muscle, and connective tissue surrounding developing

skeletal elements and tendons. Expression also was seen in the epithelium of the developing renal cortex and in spindle-shaped fibroblast-like cells in the fetal adrenal. Human WISP-1 was strongly expressed by osteoblasts at sites of bone formation in the fetal limb.

5        Human Adult Tissue The adult tissues examined were: liver, kidney, adrenal, myocardium, aorta, spleen, lung, skin, chondrosarcoma, eye, stomach, gastric carcinoma, colon, colonic carcinoma, renal cell carcinoma, prostate, bladder mucosa, and gall bladder, as well as tissue with acetaminophen-induced liver injury and hepatic cirrhosis.

No expression was seen in normal or diseased adult tissues in this study.

10        In summary, the overall pattern of expression of human WISP-1 was broadly similar to that observed for the mouse gene as noted above. The human WISP-1 probe did not cross react with the mouse embryo section.

#### Expression in Human Breast Carcinoma and Normal Breast Tissue

15        Human WISP-1 was negative on benign and malignant epithelial cells, but showed specific hybridization in mesenchymal cells, particularly in areas of tissue repair, including dystrophic ossification. Most positive cells had the morphology of fibroblasts; smooth muscle cells appeared to be negative.

20        In summary, this study shows expression of human WISP-1 RNA in mesenchymal cells involved in tissue repair and/or collagen deposition. The signal was particularly strong in benign fibroblast-like cells adjacent to either infiltrating breast carcinoma cells or tissue destruction due to benign, inflammatory conditions (duct rupture). Of note is the fact that deposition of benign osteoid seemed to correlate with strong expression of the RNA.

#### (3) Mouse WISP-2

##### Expression in Normal Mouse Tissues

25        Mouse Fetal Tissues: Expression of mouse WISP-2 was observed in osteoblasts in an E15.5 mouse embryo, within the developing mandible.

30        Mouse Adult Tissues: Expression of mouse WISP-2 was observed in stromal cells around the origin, and within the cusps of the mitral and tricuspid valves of the adult heart. Expression was also observed in the adventitial cells of the renal artery; expression was presumed to be present at this site in all arteries.

All other tissues were negative.

#### 30        Expression in Wnt-1 Tumors

The results demonstrated specific expression of mouse WISP-2 in the stroma of all Wnt-1 tumors examined. There was a signal over mononuclear cells with open vesicular nuclei, possibly macrophages. No expression was observed in either the benign or the malignant epithelium.

#### (4) Human WISP-2

##### 35        Expression in Human Tissues

Strong expression of the WISP-2-encoding gene was observed in dermal fibroblasts in normal adult skin. Additionally, strong expression was seen in two cirrhotic livers, at sites of active hepatic

fibrosis. Moderate expression was found over fasciculated cells of adrenal cortex. This localization supports a role for human WISP-2 in extracellular matrix formation or turnover.

Expression in Human Breast Carcinoma and Normal Breast Tissue, and in Lung Carcinoma

Human WISP-2 showed a similar hybridization pattern to human WISP-1 (described above) in the two breast tumors examined. It was negative on benign and malignant epithelial cells, but showed specific hybridization in mesenchymal cells, particularly in areas of tissue repair, including dystrophic ossification. The signal appeared to localize to the same cell population for both probes WISP-1 and WISP-2; however, in some areas (breast tumor 02), the signal for WISP-2 was significantly stronger than that for human WISP-1. Most positive cells had the morphology of fibroblasts; smooth muscle cells appeared to be negative. The signal for human WISP-2 was less intense in the lung tumor tissue; however, this section also showed less tissue repair compared with the breast tumor slides. Normal lung and kidney tissue were essentially negative for human WISP-2, as for human WISP-1.

In summary, this study shows expression of human WISP-2 RNA in mesenchymal cells involved in tissue repair and/or collagen deposition. The signal was particularly strong in benign fibroblast-like cells adjacent to either infiltrating breast carcinoma cells or tissue destruction due to benign, inflammatory conditions (duct rupture). Of note is the fact that deposition of benign osteoid seemed to correlate with strong expression of the RNA.

(5) Human WISP-3

Expression in Normal Adult and Fetal Tissues and in Human Breast Carcinoma and Normal Breast Tissue and in Colon Carcinoma

The analysis shows strong expression of human WISP-3 in dermal fibroblasts in normal adult skin and in cirrhotic livers at sites of active hepatic fibrosis. This localization pattern supports a role for this growth factor in extracellular matrix formation and turnover.

The probe for human WISP-3 was negative on most tissues examined. It showed a weak, diffuse positivity on sections of an osteosarcoma; some of the positive cells do represent malignant cells. WISP-3 was negative on all normal and fetal tissues examined.

EXAMPLE 17: Ability of WISP Polypeptides to Inhibit VEGF-Stimulated Proliferation of Endothelial Cell Growth

The ability of mouse and human WISP-1 and human WISP-2 polypeptides to inhibit VEGF-stimulated proliferation of endothelial cells was tested. Specifically, bovine adrenal cortical capillary endothelial (ACE) cells (from primary culture, maximum 12-14 passages) were plated on 96-well microtiter plates (Amersham Life Science) at a density of 500 cells/well per 100  $\mu$ L in low-glucose DMEM, 10% calf serum, 2 mM glutamine, 1x pen/strept, and fungizone, supplemented with 3 ng/mL VEGF. Controls were plated the same way but some did not include VEGF. A test sample of either mouse WISP-1, human WISP-1 conjugated to IgG, or human WISP-2 (PRO261) conjugated to poly-His was added in a 100- $\mu$ L volume for a 200- $\mu$ L final volume. Cells were incubated for 5-7 days at 37°C. The media were aspirated and the cells washed 1x with PBS. An acid phosphatase reaction mixture (100  $\mu$ L, 0.1 M sodium acetate, pH 5.5, 0.1% TRITON-100™, 10 mM p-nitrophenyl phosphate) was added. After incubation for 2 hours at 37°C.

the reaction was stopped by addition of 10  $\mu$ L 1 N NaOH. OD was measured on a microtiter plate reader at 405 nm. Controls were: no cells, cells alone, cells + FGF (5 ng/mL), cells + VEGF (3 ng/mL), cells + VEGF (3 ng/mL) + TGF- $\beta$  (1 ng/mL), and cells + VEGF (3 ng/mL) + LIF (5 ng/mL). (TGF- $\beta$  at a 1 ng/mL concentration is known to block 70-90% of VEGF-stimulated cell proliferation.)

5 The results were assessed by calculating the percentage inhibition of VEGF(3ng/mL)-stimulated cell proliferation, determined by measuring acid phosphatase activity at OD405 nm (1) relative to cells without stimulation, and (2) relative to the reference TGF- $\beta$  inhibition of VEGF-stimulated activity. The results, as shown in Table X below, are indicative of the utility of the WISP polypeptides in cancer therapy and specifically in inhibiting tumor angiogenesis. The numerical values (relative inhibition) shown in Table

10 X are determined by calculating the percent inhibition of VEGF-stimulated proliferation by the mouse WISP-1, human WISP-1-IgG, and human WISP-2-poly-His polypeptides relative to cells without stimulation and then dividing that percentage into the percent inhibition obtained by TGF- $\beta$  at 1 ng/mL, which is known to block 70-90% of VEGF-stimulated cell proliferation. Human WISP-1 and human WISP-2 appear to be particularly useful as angiostatic agents.

Table X

	<u>Polypeptide</u>	<u>Concentration (nM)</u>	<u>Relative Inhibition</u>
	Mouse WISP-1	0.1	113
	"	1.0	108
	"	10.0	109
20	Human WISP-1-IgG	1.1	1
	"	11.0	0.95
	"	110.0	0.9
	Human WISP-2-poly-His	0.01%	0.95
	"	0.01%	1.1
25	"	0.1	0.62
	"	1.0	1.03
	"	1.0	0.5
	"	1.0	0.6

Deposit of Material

30 The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, USA (ATCC):

	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
	pRK5E.h.WIG-1.568.38	209533	December 10, 1997
	pRK5E.m.WIG-1.568.6his	209537	December 10, 1997
35	Plasmid (encoding human WISP-2)	209391	October 17, 1997
	pRKE.m.WIG-2.1367.3	209538	December 10, 1997

DNA56350-1176-2

209706

March 26, 1998

DNA58800-1176-2

209707

March 26, 1998

5 These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures of the deposits for 30 years from the date of deposit. The deposits will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures of the deposits to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, 10 and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

15 The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited materials is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

20 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposits of materials herein do not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various 25 modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

## WHAT IS CLAIMED IS:

1. Isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) a complement of the DNA molecule of (a).  
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2. The nucleic acid of claim 1 having at least one WISP biological activity.
3. The nucleic acid of claim 1 comprising DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) a complement of the DNA molecule of (a).
- 10 4. The nucleic acid of claim 3 comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or DNA encoding a human WISP-1 polypeptide having amino acid residues 1 to 367 of Figures 3A and 3B (SEQ ID NO:4), or a complement of either of the encoding DNAs.
- 15 5. The nucleic acid of claim 3 comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 or 1 to 367 of Figures 3A and 3B except for an isoleucine residue at position 184 rather than a valine residue (SEQ ID NOS:5 and 6, respectively).
6. The nucleic acid of claim 3 comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 or 1 to 367 of Figures 3A and 3B except for a serine residue at position 202 rather than an alanine residue (SEQ ID NOS:7 and 8, respectively).
- 20 7. The nucleic acid of claim 3 comprising DNA encoding a human WISP-1 polypeptide having amino acid residues 23 to 367 or 1 to 367 of Figures 3A and 3B except for an isoleucine residue at position 184 rather than a valine residue and except for a serine residue at position 202 rather than an alanine residue (SEQ ID NOS:21 and 22, respectively).
8. Isolated nucleic acid comprising SEQ ID NO:23, 24, 25, 26, 27, 28, or 29.
- 25 9. The nucleic acid of claim 1 comprising DNA encoding a mouse WISP-1 polypeptide having amino acid residues 23 to 367 of Figure 1 (SEQ ID NO:11), or DNA encoding a mouse WISP-1 polypeptide having

amino acid residues 1 to 367 of Figure 1 (SEQ ID NO:12), or a complement of either of the encoding DNAs.

10. Isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 85% sequence identity to (a) a DNA molecule encoding a mouse WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figure 1 (SEQ ID NO:11), or (b) a complement of the DNA molecule of (a).

11. The nucleic acid of claim 10 having at least one WISP biological activity.

12. The nucleic acid of claim 10 comprising DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a mouse WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figure 1 (SEQ ID NO:11), or (b) a complement of the DNA molecule of (a).

13. Isolated nucleic acid comprising DNA having at least about 600 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-1 polypeptide cDNA in ATCC Deposit No. 209533 (pRK5E.h.WISP-1.568.38), or (b) a complement of the DNA molecule of (a).

14. A vector comprising the nucleic acid of claim 1.

15. A host cell comprising the vector of claim 14.

16. A process for producing a WISP-1 polypeptide comprising culturing the host cell of claim 15 under conditions suitable for expression of the WISP-1 polypeptide and recovering the WISP-1 polypeptide from the cell culture.

17. Isolated WISP-1 polypeptide encoded by the nucleic acid of claim 1.

18. The polypeptide of claim 17 that is human WISP-1 or mouse WISP-1.

19. Isolated WISP-1 polypeptide encoded by a nucleic acid of claim 8.

20. A chimeric molecule comprising a WISP-1 polypeptide fused to a heterologous amino acid sequence.

21. The chimeric molecule of claim 20 wherein said heterologous amino acid sequence is an epitope tag sequence, a poly-amino acid sequence, or an Fc region.
22. An antibody which specifically binds to a WISP-1 polypeptide.
23. The antibody of claim 22 wherein said antibody is a monoclonal antibody.
- 5 24. Isolated nucleic acid having at least about 600 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) a complement of the DNA molecule of (a), and, if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), isolating the test DNA molecule.
- 10 25. A polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-1 polypeptide comprising the sequence of amino acids 23 to 367 of Figures 3A and 3B (SEQ ID NO:3), or (b) a complement of the DNA molecule of (a), and if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.
- 15 26. Isolated nucleic acid comprising DNA having at least about an 80% sequence identity to (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) a complement of the DNA molecule of (a).
27. The nucleic acid of claim 26 having at least one WISP biological activity.
- 20 28. The nucleic acid of claim 26 comprising DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) a complement of the DNA molecule of (a).
- 25 29. The nucleic acid of claim 26 comprising DNA encoding a human WISP-2 polypeptide having amino acid residues 24 to 250 of Figure 4 (SEQ ID NO:15), or DNA encoding a human WISP-2 polypeptide having amino acid residues 1 to 250 of Figure 4 (SEQ ID NO:16), or a complement of either of the encoding DNAs.



30. Isolated nucleic acid comprising DNA having at least about an 80% sequence identity to (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 1 to 250 of Figure 4 (SEQ ID NO:16), or (b) a complement of the DNA molecule of (a).
- 5 31. Isolated nucleic acid comprising DNA having at least about 500 nucleotides and at least about an 80% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 24 to 251 of Figure 2 (SEQ ID NO:19), or (b) a complement of the DNA molecule of (a).
32. The isolated nucleic acid of claim 31 comprising DNA having at least about a 95% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 24 to 251 of Figure 2 (SEQ ID NO:19), or (b) a complement of the DNA molecule of (a).
- 10 33. The nucleic acid of claim 32 comprising DNA encoding a mouse WISP-2 polypeptide having amino acid residues 24 to 251 of Figure 2 (SEQ ID NO:19), or DNA encoding a mouse WISP-2 polypeptide having amino acid residues 1 to 251 of Figure 2 (SEQ ID NO:20), or a complement of either of these encoding DNAs.
- 15 34. Isolated nucleic acid comprising DNA having at least about 500 nucleotides and at least about an 80% sequence identity to (a) a DNA molecule encoding a mouse WISP-2 polypeptide comprising the sequence of amino acids 1 to 251 of Figure 2 (SEQ ID NO:20), or (b) a complement of the DNA molecule of (a).
- 20 35. Isolated nucleic acid comprising DNA having at least about 400 nucleotides and at least about a 75% sequence identity to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-2 polypeptide cDNA in ATCC Deposit No. 209391 (DNA33473), or (b) a complement of the DNA molecule of (a).
36. The nucleic acid of claim 35 comprising the nucleotide sequence of the full-length coding sequence of clone UNQ228 (DNA33473) deposited under accession number ATCC 209391.
37. A vector comprising the nucleic acid of claim 26.
38. A host cell comprising the vector of claim 37.
- 25 39. A process for producing a WISP-2 polypeptide comprising culturing the host cell of claim 38 under conditions suitable for expression of the WISP-2 polypeptide and recovering the WISP-2 polypeptide from the cell culture.

40. Isolated WISP-2 polypeptide encoded by the nucleic acid of claim 26.
41. The polypeptide of claim 40 that is isolated native-sequence human WISP-2 polypeptide comprising amino acid residues 1 to 250 of Figure 4 (SEQ ID NO:16) or comprising amino acid residues 24 to 250 of Figure 4 (SEQ ID NO:15).
- 5 42. A chimeric molecule comprising a WISP-2 polypeptide fused to a heterologous amino acid sequence.
43. An antibody which specifically binds to a WISP-2 polypeptide.
44. The antibody of claim 43 that is a monoclonal antibody.
45. Isolated nucleic acid having at least about 400 nucleotides and produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) a complement of the DNA molecule of (a), and, if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), isolating the test DNA molecule.
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46. A polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-2 polypeptide comprising the sequence of amino acids 24 to 250 of Figure 4 (SEQ ID NO:15), or (b) a complement of the DNA molecule of (a), and if the test DNA molecule has at least about a 75% sequence identity to (a) or (b), (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.
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47. Isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 500 nucleotides to (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) a complement of the DNA molecule of (a).
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48. The nucleic acid of claim 47 having at least one WISP biological activity.
49. The nucleic acid of claim 47 comprising DNA encoding a human WISP-3 polypeptide having amino acid residues 34 to 372 of Figures 6A and 6B (SEQ ID NO:32) or amino acid residues 1 to 372 of Figures 6A and 6B (SEQ ID NO:33), or a complement thereof.
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50. A vector comprising the nucleic acid of claim 47.
51. A host cell comprising the vector of claim 50.
52. A process for producing a WISP-3 polypeptide comprising culturing the host cell of claim 51 under conditions suitable for expression of the WISP-3 polypeptide and recovering the WISP-3 polypeptide from the cell culture.
53. Isolated WISP-3 polypeptide encoded by the nucleic acid of claim 47.
54. The polypeptide of claim 53 that is human WISP-3.
55. A chimeric molecule comprising the WISP-3 polypeptide of claim 53 fused to a heterologous amino acid sequence.
56. An antibody which specifically binds to the WISP-3 polypeptide of claim 53.
57. Isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 500 nucleotides to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-3 polypeptide cDNA in ATCC Deposit No. 209706 (DNA 56350-1176-2), or (b) a complement of the DNA molecule of (a).
58. Isolated nucleic acid produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) a complement of the DNA molecule of (a), and, if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 500 nucleotides, isolating the test DNA molecule.
59. A polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 34 to 372 of Figures 6A and 6B (SEQ ID NO:32), or (b) a complement of the DNA molecule of (a), and if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 500 nucleotides, (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

60. Isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 400 nucleotides to (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) a complement of the DNA molecule of (a).
- 5 61. The nucleic acid of claim 60 having at least one WISP biological activity.
62. The nucleic acid of claim 60 comprising DNA encoding a human WISP-3 polypeptide having amino acid residues 16 to 355 of Figures 7A and 7B (SEQ ID NO:36) or amino acid residues 1 to 355 of Figures 7A and 7B (SEQ ID NO:37), or a complement thereof.
- 10 63. Isolated nucleic acid comprising DNA having a 100% sequence identity in more than about 400 nucleotides to (a) a DNA molecule encoding the same full-length polypeptide encoded by the human WISP-3 polypeptide cDNA in ATCC Deposit No. 209707 (DNA 58800-1176-2), or (b) a complement of the DNA molecule of (a).
64. A vector comprising the nucleic acid of claim 60.
65. A host cell comprising the vector of claim 64.
- 15 66. A process for producing a WISP-3 polypeptide comprising culturing the host cell of claim 65 under conditions suitable for expression of the WISP-3 polypeptide and recovering the WISP-3 polypeptide from the cell culture.
67. Isolated WISP-3 polypeptide encoded by the nucleic acid of claim 60.
68. The polypeptide of claim 67 that is human WISP-3.
- 20 69. A chimeric molecule comprising the WISP-3 polypeptide of claim 67 fused to a heterologous amino acid sequence.
70. An antibody which specifically binds to the WISP-3 polypeptide of claim 67.
71. Isolated nucleic acid produced by hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) a complement of the DNA molecule of (a), and, if the test
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DNA molecule has a 100% sequence identity to (a) or (b) in more than about 400 nucleotides. isolating the test DNA molecule.

5 72. A polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a human WISP-3 polypeptide comprising the sequence of amino acids 16 to 355 of Figures 7A and 7B (SEQ ID NO:36), or (b) a complement of the DNA molecule of (a), and if the test DNA molecule has a 100% sequence identity to (a) or (b) in more than about 400 nucleotides. (ii) culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide. and (iii) recovering the polypeptide from the cell culture.

73. A composition comprising the polypeptide of claim 17 and a carrier therefor.

10 74. A composition comprising an antagonist to the polypeptide of claim 17 and a carrier therefor.

75. A composition comprising the polypeptide of claim 19 and a carrier therefor.

76. A composition comprising an antagonist to the polypeptide of claim 19 and a carrier therefor.

77. A composition comprising the polypeptide of claim 40 and a carrier therefor.

78. A composition comprising an antagonist to the polypeptide of claim 40 and a carrier therefor.

15 79. A composition comprising the polypeptide of claim 53 and a carrier therefor.

80. A composition comprising an antagonist to the polypeptide of claim 53 and a carrier therefor.

81. A composition comprising the polypeptide of claim 67 and a carrier therefor.

82. A composition comprising an antagonist to the polypeptide of claim 67 and a carrier therefor.

20 83. A composition comprising a WISP-1, WISP-2, or WISP-3 polypeptide and a pharmaceutically acceptable carrier.

84. The composition of claim 83 that further comprises a chemotherapeutic agent or growth-inhibitory agent.

85. The composition of claim 83 wherein the WISP-1, WISP-2, or WISP-3 polypeptide is a human polypeptide.

86. A pharmaceutical product comprising:

- (a) a composition of claim 83;
- 5 (b) a container containing said composition; and
- (c) a label affixed to said container, or a package insert included in said pharmaceutical product referring to the use of said WISP-1, WISP-2, or WISP-3 polypeptide in the treatment of a WISP-related disorder.

87. A process for diagnosing a disease or a susceptibility to a disease related to a mutation in a nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide comprising:

- (a) isolating a nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide from a sample derived from a host; and
- (b) determining a mutation in the nucleic acid sequence encoding a WISP-1, WISP-2, or WISP-3 polypeptide.

15 88. A method of diagnosing a WISP-related disorder in a mammal comprising detecting the level of expression of a gene encoding a WISP-1, WISP-2, or WISP-3 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample indicates the presence of a WISP-related dysfunction in the mammal from which the test tissue cells were obtained.

20 89. A method for treating a WISP-related disorder in a mammal comprising administering to the mammal an effective amount of the composition of claim 83.

90. The method of claim 89 wherein the disorder is a malignant disorder or arteriosclerosis and the mammal is human.

25 91. The method of claim 90 wherein the malignant disorder is breast cancer, ovarian cancer, colon cancer, or melanoma.

92. An isolated antibody binding a WISP-1, WISP-2, or WISP-3 polypeptide.

93. The antibody of claim 92 that induces death of a cell overexpressing a WISP-1, WISP-2, or WISP-3 polypeptide.

94. The antibody of claim 93 wherein said cell is a cancer cell.
95. The antibody of claim 92 that binds to a human WISP-1, WISP-2, or WISP-3 polypeptide, and is a human or humanized antibody.
96. The antibody of claim 92 that is a monoclonal antibody.
- 5 97. The antibody of claim 96 that is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.
98. A composition comprising an antibody of claim 92 in admixture with a pharmaceutically acceptable carrier.
99. The composition of claim 98 comprising a growth-inhibitory amount of said antibody.
- 10 100. A method for determining the presence of a WISP-1, WISP-2, or WISP-3 polypeptide comprising exposing a cell suspected of containing the WISP-1, WISP-2, or WISP-3 polypeptide to an anti-WISP-1, WISP-2, or WISP-3 antibody and determining binding of said antibody to said cell.
101. A method for treating a WISP-related disorder in a mammal comprising administering to the mammal an effective amount of a composition comprising an antagonist to a WISP-1, WISP-2, or WISP-3 polypeptide in a pharmaceutically acceptable carrier.
- 15 102. A method for inhibiting the growth of tumor cells comprising exposing a cell that overexpresses a Wnt-1-induced gene to an effective amount of an antagonist that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide.
- 20 103. A method for inhibiting the growth of tumor cells comprising exposing said cells to an effective amount of the composition of claim 99.
104. The method of claim 103 wherein the tumor cells are colon cancer cells, the antibody is against human WISP-1 and is a humanized or human monoclonal antibody, and the mammal is human.
- 25 105. A kit comprising a WISP-1, WISP-2, or WISP-3 polypeptide or antagonist and instructions for using the polypeptide or antagonist to detect or treat a WISP-related disorder.

106. The kit of claim 105 comprising an anti-WISP-1, WISP-2, or WISP-3 antibody and a carrier in suitable packaging.

107. A method for inducing cell death comprising exposing a cell that is induced by Wnt to an effective amount of a WISP-1, WISP-2, or WISP-3 polypeptide or antagonist.

5 108. An article of manufacture, comprising:

a container;

a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inducing cell death or inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overinduction of Wnt or a WISP-related disorder or by overexpression of a WISP-1, WISP-2, or WISP-3 polypeptide, and the active agent in the composition is an antagonist that inhibits the expression or activity of the WISP-1, WISP-2, or WISP-3 polypeptide.

109 The article of manufacture of claim 108 wherein the active agent is an anti-WISP-1, anti WISP-2, or anti-WISP-3 antibody, and wherein the label on the container indicates that the composition can be used for treating a WISP-related disorder.

110. A process for identifying agonists to a WISP-1, WISP-2, or WISP-3 polypeptide comprising:

(a) contacting cells and a compound to be screened under conditions suitable for the stimulation of cell proliferation by the polypeptide; and

20 (b) measuring the proliferation of the cells to determine if the compound is an effective agonist.

111. An agonist to a WISP-1, WISP-2, or WISP-3 polypeptide identified by the process of claim 110.

112. A method for identifying a compound that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide, comprising contacting a candidate compound with a WISP-1, WISP-2, or WISP-3 polypeptide under conditions and for a time sufficient to allow the compound and polypeptide to interact.

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113. The method of claim 112 comprising the steps of:

(a) contacting cells and a compound to be screened in the presence of the WISP-1, WISP-2, or WISP-3 polypeptide under conditions suitable for the stimulation of cell proliferation by polypeptide; and

(b) measuring the proliferation of the cells to determine if the compound is an effective antagonist.



114. A compound identified by the method of claim 112.

115. A compound that inhibits the expression or activity of a WISP-1, WISP-2, or WISP-3 polypeptide.

116. A method of diagnosing a WISP-related disorder in a mammal comprising (a) contacting an anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the anti-WISP-1, anti-WISP-2, or anti-WISP-3 antibody and the WISP-1, WISP-2, or WISP-3 polypeptide in the test sample.

117. The method of claim 116 wherein said test sample is obtained from an individual suspected to have neoplastic cell growth or proliferation.

1 TAACAAGGCN GTCTGTCTTG GAGAGGCATC CGCATCCTCT GGGCTGAGCC GTAGCTCCTG TGACGCTGAC TTCCAGGCAT GAGTGGCTC CTGCCCTTGA  
 ATTGTTCCGN CAGGACGAAC CTCTCCGTAG CCGTAGGAGA CCCGACTCG CACTGAGGAC ACTGCGACTG AAGTCCGTA CTCCACCGAG GACGGGACCT  
 M R W L L P W T  
 9 L A A V A V L R V G N I L A T A L S P T P T M T F T P A P L E E  
 101 CGCTGGCAGC CGTGGCAGTC CTGAGGGTGG GCAACATCCT GGCCACGGCC CTCTCTCCAA CCCCACAAC AATGACCTTC ACCCCAGCAC CACTAGAGGA  
 GCGACCGTCG GCACCGTCAG GACTCCACCC CGTTGTAGGA CCGGTGCCGG GAGAGAGTT GGGGTGTTG TTACTGGAAG TGGGTCTGTG GTGATCTCCT  
 9 L A A V A V L R V G N I L A T A L S P T P T M T F T P A P L E E  
 201 AACGACTACA CGCCCCGAAT TCTGCAAGTG GCCATGTGAG TGCCACAAT CCCCACCTCG CTGCCACTG GCGTCAAGC TAATCACAGA TGGCTGTGAA  
 TTGCTGATGT GCGGGGCTTA AGACGTTTAC CGGTACACTC ACGGTGTTA GGGGTGGAGC GACGGGTGAC CCGCAGTCGG ATTAGTGTCT ACCGACACTT  
 42 T T T R P E F C K W P C E C P Q S P P R C P L G V S L I T D G C E  
 301 TGCTGTAAGA TATGTGCCCA GCAGCTTGG GACAACTGCA CAGAGGCTGC CATCTGTGAC CCACACCGGG GCCTCTACTG CGATTACAGT GGGGATCGCC  
 ACGACATTCT ATACACGGGT CGTCGAACCC CTGTTGACGT GTCTCCGACG GTAGACACTG GGTGTGGCCC CGGAGATGAC GCTAATGTCA CCCCTAGCGG  
 75 C C K I C A Q Q L G D N C T E A A I C D P H R G L Y C D Y S G D R P  
 401 CGAGGTACGC AATAGGAGTG TGTGCACAGG TGTGCGGTGT GGGCTGTGTC CTGGATGGCG TACGCTACAC CAATGGCGAG TCCTTCCAC CCAACTGCAG  
 GCTCCATCGG TTATCCTCAC ACACGTGTCC ACCAGCCACA CCGACACAG GACCTACCGC ATCGATGTG GTTACCGCTC AGGAAGTTG GGTGACGTC  
 109 R Y A I G V C A Q V V G V G C V L D G V R Y T N G E S F Q P N C R  
 501 GTACAACTGT ACCTGCATTG ATGGCACGGT GGGTGCACA CCGTGTGCC TAAGCCCCAG GCCCCCACGC CTCTGGTGCC GCCAGCCCCG GCACGTGAGA  
 CATGTTGACA TGGACGTAAC TACCGTGCCA CCGGACGTGT GCGACACGG ATTCCGGGTC CCGGGGTGCG GAGACCACGG CCGTCGGGGC CGTGCACTCT  
 142 Y N C T C I D G T V G C T P L C L S P R P P R L W C R Q P R H V R  
 601 GTCCCTGGCC AGTGCTGTGA GCAGTGGGTG TGTGATGATG ACGCAAGGAG ACCACGCCAG ACTGCACTGT TGGACACCAG AGCCTTTGCA GCGTCAGGCG  
 CAGGGACCGG TCACGACACT CGTCACCCAC ACACACTAC TCGGTTCCCTC TGGTCCGTC TGACGTGACA ACCTGTGGTC TCGGAAACGT CGCAGTCCGC  
 175 V P G Q C C E Q W V C D D A R R P R Q T A L L D T R A F A A S G A  
 701 CCGTGGAGCA ACGGTATGAG AACTGCATAG CCTACACTAG TCCCTGGAGC CCCTGCTCTA CCACCTGTGG CCTAGGTATC TCCACTCGGA TCTCTAACGT  
 GGCACCTCGT TGCCATATC TTGACGTATC GGATGTGATC AGGGACCTCG GGGACAGAT GTTGACACC GGATCCATAG AGGTGAGCCT AGAGATTGCA  
 209 V E Q R Y E N C I A Y T S P W S P C S T T C G L G I S T R I S N V  
 801 CAATGCCCCG TGCTGGCCAG AGCAGGAAAG TCGCCTCTGC AACCTGGCGC CATGTGATGT GGACATCCAA CTACACATCA AGGCAGGGA GAAATGCTCTG  
 GTTACGGGCC ACGACCGGTC TCGTCCCTTC ACGGAGAGC TTGGACGGCG GTACACTACA CCTGTAGGTT GATGTAGT TCCGTCCCTT CTTTACGGAC  
 242 N A R C W P E Q E S R L C N L R P C D V D I Q L H I K A G K K C L

FIG. 1A

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901 GGTGTGTACC AGCCAGAGGA GGCCACGAAC TTCACCTCTCG CAGGCTGTGT CAGCACACGC ACCTACCGAC CCAAGTACTG CGGAGTCTGT ACTGACAATA  
 CGACACATGG TCGGTCTCCT CCGGTGCTTG AAGTGAGAGC GTCCGACACA GTCGTGTGCG TGGATGGCTG GGTTCATGAC GCCTCAGACA TGACTGTAT  
 275 A V Y Q P E E A T N F T L A G C V S T R T Y R P K Y C G V C T D N R  
 1001 GGTGTGTCAT CCCTACAAG TCCAAGACCA TCAGTGTGGA TTTCAGTGT CCAGAGGGGC CAGGTTTCTC CCGCAGGTC CTATGGATTA ATGCTTGCTT  
 CCACAACGTA GGGGATGTTT AGGTTCTGGT AGTCACACCT AAAGTCACA GGTCTCCCG GTCCAAAGAG GGCCGTCCAG GATACCTAAT TACGAACGAA  
 309 C C I P Y K S K T I S V D F Q C P E G P G F S R Q V L W I N A C F  
 1101 CTGCAACCTG AGTGCAGGA ATCCTAACGA TATCTTTGCT GACTTGGAT CTTACCCCTGA CTTGGAAGAG ATTGCCAATT AGGTGGGTGT GTGGCTCAGG  
 GACGTTGGAC TCGACGTCCT TAGGATTGCT ATAGAAACGA CTGAACCTTA GAATGGGACT GAAGCTTCTC TAACGGTTAA TCCACCCACA CACCGAGTCC  
 342 C N L S C R N P N D I F A D L E S Y P D F E E I A N O  
 1201 GTAAAGTTCC ATGCTGCAAA GCAGCCAGCC CTTGTGGTC CAGGACTTCA CAATTGAGCC TTATTTCAATC TACTTCTTAC TCGATTCTGA ATTCCCAGTT  
 CATTCAAGG TACGACGTTT CGTCGGTCCG GAAACACCAG GTCTGAACT GGTAACTCGG AATAAAGTAG ATGAAGGATG AGCTAAGACT TAAGGTCAA  
 1301 TCTGTTCCTG TTTTGACAAAT CGTAATGGCC CAGGAGAGTG CTGCTCAGGC TCAGACAATG GGTTCCTCCT TGGGGACATT CTACATCAAT CCAAGGAAAA  
 AGACAGGAC AAAACTGTTA GCATTACCGG GTCTCTCAC GACGAGTCCG AGTCTGTTAC CCAAGGAGGA ACCCCTGTAA GATGTAGTAA GGTTCCTTTT  
 1401 CACATCTCTG ACTGTTTACA ATGGAAGCAA AGCTGGCCC AGCTAGTCTG GCTCCAGCCT GGGCAAGTTG TCAGAAGTTG TGATGGGATT GTCCAAGGAA  
 GTGTAGAGAC TGACAAGTGT TACCTTCGTT TCGGACCCGG TCGATCAGAC CGAGGTCGGA CCCGTTCAAC AGCTTCAAC ACTACCCCTAA CAGGTTCCTT  
 1501 AAGCATCAGC TGAAGAACCA GTATCATGAA GTCCTTCCTC AGATGCCAAG CCTAGGGATG CTGGGATCCT TTCAGACAGA TGGATGGGAT TGGGGACACA  
 TTGCTAGTCG ACTTCTTGGT CATAGTACTT CAGGAAGGAG TCTACGGTTC GATCCCTTAC GACCCCTAGGA AAGTCTGTCT ACCTACCCCTA ACCCCTGTGT  
 1601 GGAATAAGCT ATTATTTTAC CCTTGCCAAA TGATACTATC CTGGGTATTT CTGCTTAAAA ACATACCAAA AGTGTTCCTG TTCCACTGAT CTGTATATCA  
 CCTTATTCGA TAATAAAATG GGAACGGTTT ACTATGATAG GACCCATAAA GACGATTTT TGTATGGTTT TCACAAGAAC AAGGTGACTA GACATATAGT  
 1701 CAAGTCACCA AACATTTTCC AGGTGAGGAC CCATAGTTGT GTCAATCTGT TTTGCCAATT GAAAAA  
 GTTCAGTGGT TTGTAAAAGG TCCACTCCTG GGTATCAACA CAGTAAGACA AAACGGTTAA CTTTTT

**FIG. 1B**

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1 CCCACGGGTC CGGCTCCTG ATCTCCAGAG GACCCCGGGC TGGACAGGG GCCTTGCGA GGCTGCACT GCTGTGGCAG TAGTTGGGA TGGAGGTCCT  
 GGGTGGCAG GCGGAGGAC TAGAGGTCTC CTGGGGCCCG ACCCTGTCCC CGGAACCGCT CCGACGTGCA CGACACCGTC ATCGAACCCCT ACCTCCAGAA  
 101 TCTTGCTGGG AACTGAGGAG CTGAGAGGCT CTTGTGAGG TCCTGTCTTA AACTCTTGGC ACTTGGGTG GCTTGGGCTT CACACACTGT CAGACACCTT  
 AGAAGACCC TTGACTCCTC GACTCTCCGA GGACAGTCCG AGGACAGGAT TTGAGAACCG TGAACGCCAC CGAACCCGAA GTGTGTGACA GTCTGTGGAA  
 201 CTTTGTGGCC TCCTCGGCCT CAGGTTTGA GGTGGCTCCA CAAGGGACAC GGTGACATGA GGGGCAACCC ACTGATCCAT CTCTGGCCA TTTCTTCTCT  
 GAACCAACCG AGGAGCCGA GTCCAAACTT CGACCCGAGT GTTCCCTGTG CCACTGTACT CCCCCTTGGG TGACTAGGTA GAAGACCGGT AAAGGAAGGA  
 1 M R G N P L I H L L A I S F L  
 301 CTGCAATTCTC TCAATGGTGT ATTCCAGCT GTGCCAGCA CCCTGTGCCT GTCCTTGGAC ACCACCCAG TCCCCACCG GGTACCCCT GGTGCTGGAT  
 GACGTAAGAG AGTTACCACA TAAGGTCTGA CACGGTCTGT GGGACACGGA CAGGAACCTG TGGTGGGTG ACGGTGGCC CCCATGGGA CCACGACCTA  
 16 C I L S M V Y S Q L C P A P C A C P W T P P Q C P P G V P L V L D  
 401 GGCTGTGGCT GCTGTGAGT GTGTGACGG AGGCTGGGG AGTCTGCGA CCACCTGCAT GTCTGCGACC CCAGCCAGG CCTGGTTGT CAGCCTGGG  
 CCGACACCGA CGACAGCTCA CACAGTGCC TCCGACCCCC TCCAGACGCT GGTGACGTA CAGACGCTG GGTGCTGCC GGACCAAACA GTCGGACCCC  
 49 G C G C C R V C A R R L G E S C D H L H V C D P S Q G L V C Q P G A  
 501 CAGGCCCAG TGGCCGTGGT GCTGTGACC TCTTCAAGA GGATGACGG AGCTGTGAG TGAATGGCC CAGGTACCTG GATGGGGAGA CCTTAAACC  
 GTCCGGGTC ACCGGACCA CGACACAGG AGAAGTTCT CTTACTGCCC TCGACACTCC ACTTACCGC GTCCATGGAC CTACCCCTCT GGAATTTGG  
 83 G P S G R G A V C L F E E D D G S C E V N G R R Y L D G E T F K P  
 601 CAATTGCAGG GTTTTGTGCC GCTGTGATGA CGGTGTTTC ACCTGCTGC CGTGTGCGA TGAGGATGT CGGTGCCA GCTGGGACTG CCCACGCCCC  
 GTTAACGTC CAAACACAG CGACACTACT GCCACCAAAG TGGACGGACG GCACACGTC ACTCCTACAC GCCGACGGT CGACCTGAC GGGTGGGGG  
 116 N C R V L C R C D D G G F T C L P L C S E D V R L P S W D C P R P  
 701 AGGAGAATAC AGGTGCCAGG AAGTGCTGC CCGAGTGGG TGTGTGACCA GGCAGTGATG CAGCCGGCAA TCCAGCCCTC CTCAGCCCAA GGACACCAAC  
 TCCCTTTATG TCCACGGTCC TTCCACGACG GGGCTCACC ACACACTGGT CCGTCACTAC GTCCGCCGT AGGTGGGAG GAGTCGGGT CCTGTGGTTG  
 149 R R I Q V P G R C C P E W V C D Q A V M Q P A I Q P S S A Q G H Q L  
 801 TTTCTGCCCT GTCACTCCT GCATCTGCC ATGGCCCTG TCCAAACTGG AGCAGACCT GGGGCCCTG CTCACACACC TGTGGTTGG GCATAGCCAC  
 AAAGACGGGA ACAGTGAGGA CGTAGACGGC TACCGGGGAC AGTTTGACC TCGTGTGGA CCCCCGGAC GAGTTGGTG ACACCAACC CGTATCGGTG  
 183 S A L V T P A S A D G P C P N W S T A W G P C S T T C G L G I A T  
 901 CCGAGTATCC AACCAGAAC GATTCTGCCA ACTGGAGATC CAGCGTCGCC TGTGTCTGTC CAGACCTGC CTGGCATCCA GGAGCCACGG CTCATGGAAC  
 GGCTCATAGG TTGGTCTTGG CTAAGACGGT TGACCTCTAG GTCGACGGG ACACAGACAG GTCTGGGACG GACCGTAGGT CCTCGGTGCC GAGTACCTTG  
 216 R V S N Q N R F C Q L E I Q R R L C L S R P C L A S R S H G S W N

FIG.-2A

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1001 AGTGCTTCT AGAGCCATTG CGGGATGTG GATACAGGGC CTGCCATTCT CAGCAATGT CCCTAGGACC AGCCCTTGA CTGATGGTAG ATGCCCTCT
    TCACGGAAGA TCTCGGTAAC GCCCCATAC CTATGTCCG GACGGTAAGA GTCGTTTACA GGGATCCTGG TCCGGGACCT GACTACCATC TACGGGGAGA
249 S A F O
1101 CCATGCTCTT GGTGCAGTT AACTGTCTCT GGTGATTCA GTGTCCAGAG CCTCTGAGCG ATCCCTGCTC TGTCTGAGGT GGGGAAGCA GGTGACCAGC
    GGTACGAGAA CCGACGTCAA TTGACAGGAC CCACCTAAGT CACAGGTCTC GGAGACTCG TAGGACGAG ACAGACTCCA CCCCCTTCGT CCACTGGTCG
1201 TCCATTCTC TGAATCTGA CCAGGCTTC TGGGTCTCC TGGCTAGTTC CTCAAAACTT CCCTGTATGA AAAGGACAAC CAAAAGGACC TTAAAAGCTA
    AGGTAAAGAG ACCTAAGACT GGTCCGAAG ACCCAAGAG ACCGATCAAG GAGTTTGA GGGACATACT TTTCCTGTTG GTTTTCTCGG AAATTTTCGAT
1301 AGCTGTACTG GGCAGCCTG GCCACCATGC TGGGATAGT GACAGTAATA GGTACCAGGC AGCAGATTGC CTGAAACATC CAGGTCCCTT CTTGGACTTC
    TCGACATGAC CCGTTCCGAC CGGTGGTACG ACCCTATCA CTGTCATTAT CCATGGTCCG TCGTCTAACG GACTTTGTAG GTCCAGGGAA GAACCTGAAG
1401 TATGTGCTTG TCCCAAAGAT TATGGGTGAC TATGTAAAGT TGCCTTTCCT GATCTGAGAA CACCCTGCCC GGCTGGGAG AATTTTCTGG GAACATGAAG
    ATACACGAAC AGGTTTCTA ATACCCACTG GAACATTCAC ACGGAAAGGA CTAGACTCTT GTGGGACGGG CCGACCCCTC TTAAAAGACC CTTGTACTTC
1501 AGATGGAATC ACATATTCT TAAGAGCGTT TGCCAAGTCC AGGAACCTGA CCTTTGTATT TGTAAAAATA CACATCTCTT AAATGCTCAC AAAGCAAGAG
    TCTACCTTAG TGTGATAAGA ATTCTCGCA ACGGTTCAAG TCCTTGAAC TCGAAACATA ACATTTTAT GTGTAGAGAA TTTACGAGTG TTTCTGTTCTC
1601 GCTCCACACT TCTGGCAGGC CAGGCTCTT CTCTCAGCA TGAGAGAGAC AAGAACAGT AGAGTACCCT CCTCTGGAG ACTGGCCCGG TCTGGAATAA
    CGAGGTGTGA AGACCGTCCG GTCCCGGAAA GAGAAGTCGT ACTCTCTCTG TTCCTTGTCA TCTCATGGGA GGAGACCTCC TGACCGGGCC AGACCTTATT
1701 ACACCCAAAT CAAGTGTGGA AAAAAAAA AAAA
    TGTGGGTTA GTTCACACCT TTTTTTTTTT TTTT

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**FIG. 2B**

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1 CCCACGGTC CGTGGGCC AGCTCCCCG AGAGGTGGT GATCCTCTG GGCTGCTCG TGCCTACTG TGCCACTGAC GTCCAGGCAT GAGTGGTTC  
 GGTGGCAG GCGACCCGG TCGAGGGGC TCTCCACCAG CCTAGGAGC CCGACGAGC AGCTACGAC ACGTGACTG CAGGTCCGTA CTCCACCAAG  
 M R W F  
 1  
 101 CTGCCCTGGA CGTGGCAGC AGTGACAGCA GCAGCCGCCA GCACCGTCCT GGCCACGGCC CTCTCTCCAG CCCCTACGAC CATGGACTTT ACTCCAGTTC  
 GACGGACCT GCGACCGTCG TCACTGTCTG CTGCGCGGT CGTGGCAGGA CCGGTGCCG GAGAGAGTC GGGGATGCTG GTACCTGAAA TGAGGTGAG  
 5 L P W T L A A V T A A A A S T V L A T A L S P A P T T M D F T P A P  
 201 CACTGGAGGA CACCTCCTCA CGCCCCCAAT TCTGCAAGTG GCCATGTGAG TGCCCGCCAT CCCACCCCG CTGCCCGCTG GGGTCAGCC TCATCACAGA  
 GTGACTCCT GTGAGGAGT GCGGGGTTA AGACGTTTAC CGGTACTC CCGTACACTC ACGGCGGTA GGGGTGGGC GACGGGCGAC CCCACGTCG AGTAGTCT  
 39 L E D T S S R P Q F C K W P C E C P S P P R C P L G V S L I T D  
 301 TGGCTGTGAG TGTGCTAAGA TGTGCGCTCA GCAGCTTGG GACAACTGCA CGGAGGTGC CATCTGTGAC CCCCACCGG GCCTCTACTG TGACTACAGC  
 ACCGACACTC ACGACATCT ACACGGAGT CGTGAACCC CTGTTGACGT GCCTCCGACG GTAGACACTG GGGTGGGCC CGGAGATGAC ACTGATGTCG  
 72 G C E C C K M C A Q Q L G D N C T E A A I C D P H R G L Y C D Y S  
 401 GGGGACCGCC CGAGGTACGC AATAGGAGTG TGTGCACAGG TGGTCGGTGT GGGCTGCTC CTGGATGGG TGCGCTACAA CAACGGCCAG TCCTTCCAGC  
 CCCGTGGCG GCTCCATGCG TTATCTCTAC ACAGTGTCC ACCAGCCACA CCCGACGCG GACCTACCC ACGGATGTT GTTGCCGGTC AGGAAGTTCG  
 105 G D R P R Y A I G V C A Q V V G V G C V L D G V R Y N N G Q S F Q P  
 501 CTAACCTGCAA GTACACTGC ACGTGCATCG ACGCGCGGT GGGCTGCACA CCACTGTGCC TCCGAGTGG CCCCCCGCT CTCTGGTGCC CCCACCCGCG  
 GATTGACGT CATGTTGACG TGCACGTAGC TGCCCGGCCA CCGACGTGT GGTGACACG AGGCTCACG GGGGGCGCA GAGACCACG GGGTGGCGC  
 139 N C K Y N C T C I D G A V G C T P L C L R V R P P R L W C P H P R  
 601 GCGCGTGAGC ATACCTGGCC ACTGCTGTGA GCAGTGGTA TGTGAGGACG AGCCCAAGAG GCCACGCAAG ACCGACCCC GTGACACAGG AGCCTTCGAT  
 CGCGCACTCG TATGGACCG TGACGACACT CGTCAACCAT AACTCCTGC ACEDD A K R P R K T A P R D T G A F D  
 172 R V S I P G H C C E Q W V C E D D A K R P R K T A P R D T G A F D  
 701 GCTGTGGTG AGGTGGAGC ATGGACAGG AACTGCATAG CCTACACAAG CCCCTGGAGC CCTTGCTCCA CCAGTGGC CCTGGGGGTC TOCACTCGGA  
 CGACACCCAC TCCACCTCG TACCGTGTCC TTGACGTATC GGATGTGTTT GGGACCTCG GGAACGAGT GGTGACGCG GACCCCCAG AGGTGAGCCT  
 205 A V G E V E A W H R N C I A Y T S P W S P C S T S C G L G V S T R I  
 801 TCTCCAATGT TAACGCCCAG TGCTGGCCTG AGCAAGAGAG CCGCCTCTGC AACTTGGCG CATGCGATGT GGACATCCAT ACACATCATT AGGCAGGGAA  
 AGAGTTACA ATTGCGGTC ACGACCGGAC TCGTTCTCTC GCGGAGACG TTGAACGCC GTACGTACA CCTGTAGTA TGTAGTAAT TCCGTCCCTT  
 239 S N V N A Q C W P E Q E S R L C N L R P C D V D I H T L I K A G K

**FIG.\_3A**

901 GAAGTGTCTG GCTGTGTACC AGCCAGAGGC ATCCATGAAC TTCACACTTG CGGCTGCAT CAGCACACGC TCCTATCAAC CCAAGTACTG TGGAGTTTGC  
 CTTACACAGC CGACACATGG TCGGTCTCCG TAGGTACTTG AAGTGTGAAC GCCGACGTA GTCTGTGCG AGGATAGTTG GGTTCATGAC ACCTCAACAG  
 272 K C L A V Y Q . P E A S M N F T L A G C I S T R S Y Q P K Y C G V C  
 1001 ATGGACAATA GGTGCTGCAT CCCCTACAAG TCTAAGACTA TCGACGTGTC CTTCCAGTGT CCTGATGGGC TTGCTTCTC CGCCAGGTC CTATGGATTA  
 TACCTGTTAT CCACGACGTA GGGGATGTTT AGATTCTGAT AGCTGCACAG GAAGTGCACA GGACTACCCG AACGGAAGAG GCGGTGCCAG GATACCTAAT  
 305 M D N R C C I P Y K S K T I D V S F Q C P D G L G F S R Q V L W I N  
 1101 ATGCCTGCTT CTGTAACCTG AGCTGTAGGA ATCCCAATGA CATCTTTGCT GACTTTGAAAT CCTACCCTGA CTCTCAGAA ATTGCCAACT AGGCAGGCAC  
 TACGGACGAA GACATTGGAC TCGACATCCT TAGGGTTACT GTAGAAACGA CTGAACCTTA GGATGGGACT GAAGATCTT TAACGGTTGA TCCGTCCGTG  
 339 A C F C N L S C R N P N D I F A D L E S Y P D F S E I A N O  
 1201 AAATCTTGGG TCTTGGGGAC TAACCCCAATG CCTGTGAAGC AGTCAGCCCT TATGGCCAAAT AACTTTTCAC CAATGAGCCT TAGTTACCCT GATCTGGACC  
 TTTAGAACCC AGAACCCCTG ATTGGGTTAC GGACACTTCG TCAGTCGGGA ATACCCGTTA TTGAAGAGTG GTTACTCGGA ATCAATGGGA CTAGACCTGG  
 1301 CTGGCCCTCC ATTTCTGTCT CTAACCATTC AAATGACGCC TGATGGTGCT GCTCAGGCC ATGCTATGAG TTTTCTCCTT GATATCATTC AGCATCTACT  
 GAACCGGAGG TAAAGACAGA GATTGGTAAG TTTACTGCGG ACTACCAGA CGAGTCCGGG TACGATCTC AAAAGAGAA CTATAGTAAG TCGTAGATGA  
 1401 CTAAAGAAAA ATGCCTGTCT CTAGCTGTTT TGGACTACAC CCAAGCCTGA TCCAGCCTTT CCAAGTCACT AGAAGTCTTG CTGATCTTG CCTAAATCCC  
 GATTCTTTT TACGGACAGA GATCGACAAG ACCTGATGT GGTTCGGACT AGTTCGGAAA GGTTCAGTGA TCTTCAGGAC GACCTAGAAC GGATTTAGGG  
 1501 AAGAAATGGA ATCAGGTAGA CTTTAAATAT CACTAAATTC TTCTTTAGAT GCCAAACCAC AAGACTCTTT GGGTCCATTC AGATGAATAG ATGGAATTTG  
 TTCTTTACCT TAGTCCATCT GAAATTTATA GTGATTAAG AAGAAATCTA CGGTTTGGTG TTCTGAGAAA CCCAGGTAAG TCTACTTATC TACCTTAAAC  
 1601 GAACAATAGA ATAACTATTT ATTTGGAGCC TGCCAAAGAG TACTGTAATG GGTAATTTCT AGTCAATAC CCATTAAGAC TGCAGTCGCG TGGTTTGTAT AGGACTAAGG TTTATACATA  
 CTTGTTATCT TATTAGATAA TAAACCTCGG ACGGTTCTCC ATGACATTAC CCATTAAGAC TGCAGTCGCG TGGTTTGTAT AGGACTAAGG TTTATACATA  
 1701 GCACCTCAAG GTCATCAAC ATTTGCCAAG TGAGTTGAAT AGTTGCTTAA TTTTGAATTT TAATGGAAAG TTGTATCCAT TAACTGGGC ATTGTTGAGG  
 CGTGGAGTTC CAGTAGTTTG TAAACGGTTC ACTCAACTTA TCAACGAATT AAAACTAAA ATTACCTTTC AACATAGGTA ATTGGACCCG TAACAACCTCC  
 1801 TTAAGTTTCT CTTACCCCTT ACACCTGTGA GGTACAGAT TAGGTTTGTG CCAGTCAGAA ATAAATTTG ATAAACATTC CTGTTGATGG GAAAAGCCCC  
 AATTCAAAGA GAAGTGGGA TGTGACACTT CCCATGTCTA ATCCAAACAG GGTCACTCTT TATTTTAAAC TATTTGTAG GACAACCTACC CTTTTCGGGG  
 1901 CAGTTAATAC TCCAGAGACA GGGAAAGGTC AGCCCATTTT AGAAGGACCA ATTGACTCTC ACACCTGAATC AGCTGCTGAC TGGCAGGGCT TTGGGCAGTT  
 GTCAATTATG AGGTCTCTGT CCCTTTCCAG TCGGTAAG TCTTCTCTGT TAACTGAGAG TGTGACTTAG TCGACGACTG ACCGTCCCGA AACCCGTCAA

**FIG.-3B**

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2001 GGCAGGCTC TTCCTTGAAT CTTCTCCCTT GTCCTGCTTG GGTTCATAGG AATTGGTAAG GCCTCTGGAC TGGCCTGTCT GGGCCCTGAG AGTGTGCCCC
    CCGTCCGAG AAGGAACCTTA GAAGAGGGAA CAGGACGAAC CCAAGTATCC TTAACCATTC CGGAGACCTG ACCGGACAGA CCGGGGACTC TCACCACGGG

2101 TGGAACACTC CTCTACTCTT ACAGAGCCTT GAGAGACCCA GCTGCAGACC ATGCCAGACC CACTGAAATG ACCAAGACAG GTTCAGGTAG GGGTGTGGT
    ACCTGTGTGAG GAGATGAGAA TGCTCTGGAA CTCTCTGGGT CGACGTCTGG TACGGTCTGG GTGACTTTAC TGGTTCTGTC CAAGTCCATC CCCACACCCA

2201 CAAACCAAGA AGTGGGTGCC CTTGGTAGCA GCCTGGGGTG ACCTCTAGAG CTGGAGGCTG TGGGACTCCA GGGGCCCCG TGTTCAGGAC ACATCTATTG
    GTTTGGTTCT TCACCCACGG GAACCATCGT CGGACCCAC TGGAGATCTC GACCTCCGAC ACCCTGAGGT CCCCAGGGGC ACAAGTCCCTG TGTAGATAAC

2301 CAGAGACTCA TTTCACAGCC TTTCGTTCTG CTGACCAAAT GGCAGTTTT CTGGTAGGAA GATGGAGGT TACCAGTTGT TTAGAAACAG AAATAGACTT
    GTCTCTGAGT AAAGTGTCTG AAAGCAAGAC GACTGGTTTA CCGTCAAAA GACCATCCTT CTACCTCCAA ATGGTCAACA AATCTTTGTC TTTATCTGAA

2401 AATAAAGGTT TAAAGCTGAA GAGTTGAAG CTAAAAAGAA AAGTTGTTG TTAATGAATA TCAGGCTATT ATTTATTGTA TTAGGAAAAA ATAATATTTA
    TTATTTCCAA ATTTCCGACTT CTCCAACCTC GATTTTCCTT TTCCAACAAC AATTACTTAT AGTCCGATAA TAAATAACAT AATCCTTTTA TATTATAAAT

2501 CTGTTAGAAT TCTTTTATTT AGGCCTTTT CTGTGCCAGA CATTGCTCTC AGTGCTTTGC ATGTATTAGC TCACTGAATC TTCACGACAA TGTGAGAAG
    GACAATCTTA AGAAATAAAA TCCCGGAAAG GACACGGTCT GTAACGAGAG TCACGAAACG TACATAATCG AGTGACTTAG AAGTGCTGTT ACAACTCTTC

2601 TTCCCATAT TATTCTGTT CTTACAAAATG TGAACGGAA GCTCATAGAG GTGAGAAAAA TCACCCAGT TCACCCAGT GGTGACTGG AAAGTTAGGA
    AAGGGTAATA ATAAAGACAA GAATGTTTAC ACTTTGCCCT CGAGTATCTC CACTCTTTTG AGTTGGTCTC AGTGGGTCAA CCACTGACCC TTCAATCCT

2701 TTCAGATCGA AATTGGACTG TCTTTATAAC CCATATTTTC CCCTGTTTT TAGAGCTTCC AAATGTGTCA GAATAGGAAA ACATTGCAAT AAATGGCTTG
    AAGTCTAGCT TTAACCTGAC AGAATATTG GGTATAAAG GGGACAAAA ATCTCGAAGG TTACACAGT CTATCCTTT TGTAAACGTTA TTTACCGAAC

2801 ATTTTTTAAA AAAAAAAA AAAAAAAA
    TAAAAAATTT TTTTTTTTTT TTTTTTTTTT

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**FIG.\_3C**



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1 CCCACGGC CGCTGGGA CATGAGAGC ACACCGAAGA CCCACCTCTT GGCCTTCTCC CTCTCTGCC TCCTCTCAA GGTGCGTACC CAGCTGTGCC  
 GGGTGGCAG GCCGACCCCT GTACTCTCCG TGTGGCTTCT GGTGGAAGA CCGAAGAGG GAGGACCG AGGAGAGTTT CCACGCATGG GTCGACACGG  
 1 M R G T P K T H L L A F S L L C L L S K V R T Q L C P  
 101 CGACACCATG TACCTGCCCC TGGCCACCTC CCCGATGCC GCTGGGAGTA CCCCTGGTGC TGGTGGCTG TGGTGTGTC CGGTATGTG CACGGCGGCT  
 GCTGTGGTAC ATGGACGGG ACCGGTGGAG GGGCTACGG CGACCTCAT GGGGACCACG ACCTACCGAC ACCGACGACG GCCATACAC GTGCCGCCGA  
 28 T P C T C P W P P P R C P L G V P L V L D G C G C C R V C A R R L  
 201 GGGGAGCCC TGGACCAAC TCCACGTCTG CGACGCCAGC CAGGGCCTGG TCTGCCAGCC CGGGCAGGA CCCGTGGCC GGGGGCCCT GTGCCCTCTG  
 CCCCCTCGG ACGCTGGTGT AGGTGCAGAC GCTGCGGTG GTCCCGGACC AGACGGTGG GCCCCCTCTT GGGCCACCG CCCCCCGGA CACGGAGAAC  
 61 G E P C D Q L H V C D A S Q G L V C Q P G A G P G R G A L C L L  
 301 GCAGAGGACG ACAGCAGCTG TGAGGTGAAC GGCCGCCCTGT ATCGGAAGG GGAGACCTTC CAGCCCACT GCAGATCCG CTGCCCTGC GAGGACGGCG  
 CGTCTCCTGC TGTGTCGAC ACTCCACTTG CCGGCGGACA TAGCCCTTCC CCTCTGGAAG GTCGGGTGA CGTCGTAGGC GACGGCGACG CTCCTGCCGC  
 94 A E D D S S C E V N G R L Y R E G E T F Q P H C S I R C R C E D G G  
 401 GCTTCACTG CGTCCCGCTG TGCAGCGAGG ATGTGCGGT GCCCAGCTGG GACTGCCCC ACCCCAGAG GGTGCGAGTC CTGGCAAGT GCTGCCCTGA  
 CGAAGTGGAC GCACGGCGAC ACGTCGCTCC TACACGCCGA CCGGTGACC CTGACGGGG TGGGTCTC CCAGCTCCAG GACCCGTCA CGACGGGACT  
 128 F T C V P L C S E D V R L P S W D C P H P R R V E V L G K C C P E  
 501 GTGGGTGTG GGCACAGGAG GGGACTGGG GACCCAGCCC CTTCCAGCCC AAGACCCCA GTTTCTTGGC CTTGTCTCTT CCCTGCCCCC TGGTGTCCCC  
 CACCCACACG CCGGTTCTC CCGCTGACCC CTGGGTCCGG GAAGTCCGG TTCCTGGGT CAAAGACCG GAACAGAGAA GGGACGGGG ACCACAGGGG  
 161 W V C G Q G G L G T Q P L P A Q G P Q F S G L V S S L P P G V P  
 601 TGCCAGAAAT GGAGCAGGC CTGGGACCC TGCTCGACCA CCTGTGGGT GGGCATGGC ACCCGGTGT CCAACAGAA CCGTCTCTGC CGACTGGAGA  
 ACGGTCTTA CCTCGTCCG GACCCCTGG ACAGCTGGT GGACACCCGA CCCGTACCG TGGGCCACA GGTGTGTCTT GGCGAAGACG GCTGACCTCT  
 194 C P E W S T A W G P C S T T C G L G M A T R V S N Q N R F C R L E T  
 701 CCCAGCGCG CCTGTGCTG TCCAGGCCCT GCCACCCCTC CAGGGTGC AGTCCACAA ACAGTGCCTT CTAGAGCCG GCTGGGAATG GGGACACGGT  
 GGGTCGGGC GGACACGGAC AGGTCCGGA CCGGTGGAG GTCCCGAG GTAGGTGTTT TGTACGGAA GATCTCGGC CGACCTTAC CCCTGTGCCA  
 228 Q R R L C L S R P C P P S R G R S P Q N S A F O  
 801 GTCCACCATC CCCAGCTGGT GGCCCTGTG CTGGGCCCTG GGCTGATGA AGATGTCG TGCCCCAGCC CTTGGCTGCA GGCAACACTT TAGCTTGGGT  
 CAGGTGGTAG GGTGCGACA CCGGGACACG GACCCGGGAC CCGACTACCT TCTACCAGG ACGGGTCCG GAACCGACGT CCGTGTGAA ATCGAACCA

**FIG. 4A**

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901 CCACCATGCA GAACACCAAT ATTAACACCC TGCCTGGTCT GTCTGGATCC CGAGGTATGG CAGAGGTGCA AGACCTAGTC CCCTTTCCTC TAACTCACTG  
GGTGGTACGT CTTGTGGTTA TAATTGTGCG ACGGACCCAGA CAGACCTAGG GCTCCATACC GTCTCCACGT TCTGGATCAG GGGAAAGGAG ATTGAGTGAC

1001 CCTAGGAGGC TGGCCCAAGGT GTCCAGGGTC CTCTAGCCCA CTCCCTGCCT ACACACACAG CCTATATCAA ACATGCACAC GGGCGAGCTT TCTCTCCGAC  
GGATCCTCCG ACCGGTTCCA CAGGTCCCAG GAGATCGGGT GAGGGACGGA TGTGTGTGTC GGATATAGTT TGTACGTGTG CCCGCTCGAA AGAGAGGCTG

1101 TTCCCTCTGG CAAGAGATGG GACAAGCAGT CCTTTAATAT TGAGGCTGCA GCAGGTGCTG GGCTGGACTG GCCATTTTTC TGGGGGTAGG ATGAAGAGAA  
AAGGGGACCC GTTCTCTACC CTGTTCTGTC GGAATTATA ACTCCGACGT CGTCCACGAC CCGACCTGAC CGGTAAAAAG ACCCCCATCC TACTTCTCTT

1201 GGCACACAGA GATTCTGGAT CTCCTGCTGC CTTTCTGGA GTTTGTAAAA TTGTTCTCTGA ATACAAGCCT ATGCGTGAAA AAAAAAAA AAA  
CCGTGTGTCT CTAAGACCTA GAGGACGACG GAAAAGACCT CAAACATTTT AACCAAGGACT TATGTTCCGA TACGCACTTT TTTT

**FIG. 4B**

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1 5' -CTGCAGGGGACATGAGAGGCACACCGAAGACCCACCTCCTGGCCTTCTC  
51 CCTCCTCTGCCTCCTCTCAAAGGTGCGTACCCAGCTGTGCCCGACACCAT  
101 GTACCTGCCCCTGGCCACCTCCCCGATGCCCGCTGGGAGTACCCCTGGTG  
151 GTGGATGGCTGTGGCTGCTGCCGGGTATGTGCACGGCGGCTGGGGGAGCC  
201 CTGCGACCAACTCCACGTCTGCGACGCCAGCCAGGGCCTGGTCTGCCAGC  
251 CCGGGGCAGGACCCGGTGGCCGGGGGGCCCTGTGCCTCTTGGCAGAGGAC  
301 GACAGCAGCTGTGAGGTGAACGGCCGCCTGTATCGGGAAGGGGAGACCTT  
351 CCAGCCCCACTGCAGCATCCGCTGCCGCTGCGAGGACGGCGGCTTACCT  
401 GCGTGCCGCTGTGCAGCGAGGATGTGCGGCTGCCCAGCTGGGACTGCCCC  
451 CACCCCAGGAGGGTCGAGGTCCTGGGCAAGTGCTGCCCTGAGTGGGTGTG  
501 CGGCCAAGGAGGGGGACTGGGGACCAGCCCTTCCAGCCCAAGGACCCC  
551 AGTTTTCTGGCCTTGTCTCTTCCCTGCCCCCTGGTGTCCCCTGCCCAGAA  
601 TGGAGCACGGCCTGGGGACCCTGCTCGACCACCTGTGGGCTGGGCATGGC  
651 CACCCGGGTGTCCAACCAGAACCGCTTCTGCCGACTGGAGACCCAGCGCC  
701 GCCTGTGCCTGTCCAGGCCCTGCCACCCTCCAGGGGTGCGAGTCCACAA  
751 AACAGTGCCTTCTAGAGCCGGGCTGGGAATGGGGACACGGTGTCCACCAT  
801 CCCCAGCTGGTGGCCCTGTGCCTGGGCCCTGGGCTGATGGAAGA

**FIG.\_5**

```

1 GTGGGGTTTCAGAGGAGACAGGGAGCTTTGTGTACCCGGAGCAATGAACAAGCGGGGACTTCTCTACC
  CACCCCAACGTCCTCCTCTGTCTCCCTCGAACAACATGGGCCCTCGTTACTTGTTCGCCGCTGAAGAGATGG
    M N K R R L L Y P
1
71 CCTCAGGGTGGCTCCACGGTCCCAGCGACA
  GGAGTCCCAACGAGGTGCCAGGGTCGCTGT
10 S G W L H G P S D M
101 TGCAGGGGCTCCTCTTCTCCACTCTTCTGCTGGCCTGGCACAGTTCTGCTGCAGGGTACAGGGCAC
  ACGTCCCCGAGGAGAGAGGTGAGAAGACGACCGGACCGTGTCAAGACGAGCTCCCATGTCCCCGTG
20 Q G L L F S T L L L A G L A Q F C C R V Q G T
171 TGGACCATTAGATACAAACACCTGAAGGAAG
  ACCTGGTAATCTATGTTGTGGACTTCCTTC
43 G P L D T T P E G R
201 GCCTGGAGAAGTGTGATGCACCTCAGCGTAAACAGTTTTTGTCACTGGCCCTGCAAAATGCCCTCAGCAG
  CGGACCTCTTCACAGTCTACGTGGAGTCGATTTGTCAAACAGTAGCCGGGACGTTTACGGGAGTCGTC
53 P G E V S D A P Q R K Q F C H W P C K C P Q Q
271 AAGCCCCGTTGCCCTCCTGGAGTGAGCCTG
  TTCGGGGCAACGGGAGGACCTCACTCGGAC
76 K P R C P P G V S L
301 GTGAGAGATGGCTGTGGATGCTGTAAATCTGTGCCAAGCAACGAGGGAATCTGCAATGAAGCTGACC
  CACTCTTACCGACACCTACGACATTTAGACACGGTTCGTTGGTCCCTTTAGACGTTACTTCGACTGG
86 V R D G C G C C K I C A K Q P G E I C N E A D L
371 TCTGTGACCCACAAAGGGCTGTATTGTG
  AGACACTGGGTGTGTTTCCCGACATAACAC
110 C D P H K G L Y C D
401 ACTACTCAGTAGACAGGCCCTAGGTACGAGACTGGAGTGTGTGCATACCTTGTAGCTGTTGGGTGCGAGTT
  TGATGAGTCATCTGTCCGGATCCATGCTCTGACCTCACACACGATGGAACATCGACAACCCACGCTCAA
120 Y S V D R P R Y E T G V C A Y L V A V G C E F
471 CAACCAAGGTACATTATCATTAATGGCCAAGT
  GTTGGTCCATGTAATAGTATTACCGGTTCA
143 N Q V H Y H N G Q V

```

**FIG.\_6A**

501 GTTTCAGCCCAACCCCTTGTTTCAGCTGCCTCTGTGTGAGTGGGGCCATTGGATGCACACCTCTGTTCATA  
CAAAGTCGGGTGGGGAACAAGTCGACGGAGACACACTCACCCGGTAACCTACGTGTGGAGACAAAGTAT  
153 F Q P N P L F S C L C V S G A I G C T P L F I  
571 CCAAAGCTGGCTGGCAGTCACCTGCTCTGGA  
GGTTTCGACCGACCGTCAGTGACGAGACCT  
176 P K L A G S H C S G  
601 GCTAAAGGTGGAAGAAGTCTGATCAGTCAAACTGTAGCCTGGAACCATTAATACAGCAGCTTTCAACAA  
CGATTTCCACCTTTCTTCAGACTAGTCAGTTGACATCGGACCTTGGTAATGATGTCGTCGAAAGTTGTT  
186 A K G G K K S D Q S N C S L E P L L Q Q L S T S  
671 GCTACAAAACAATGCCAGCTTATAGAGATC  
CGATGTTTTGTACGGTCGAAATATCTCTAG  
210 Y K T M P A Y R D L  
701 TCCCACTTATTTGGAAAAAATGCTTTGTGCAAGCAACAAATGGACTCCCTGCTCCAGAAACATGTGG  
AGGTGAATAAACCTTTTTTTTACAGAACACGTTCTGTTGTTTACCTGAGGACGAGGCTGTGTACACC  
220 P L I W K K K C L V Q A T K W T P C S R T C G  
771 GATGGGAATATCTAACAGGGTGACCAATGA  
CTACCCCTTATAGATTGTCCCACTGGTTACT  
243 M G I S N R V T N E  
801 AAACAGCAACTGTGAAATGAGAAAAAGAGAAAGACTGTGTTACATTCAGCCTTGGACAGCAATATATTA  
TTTGTGCTTGACACTTTACTCTTTTCTCTTTTCTGACACAAATGTAAGTCGGAACGCTGTCGTTATATAAT  
253 N S N C E M R K E K R L C Y I Q P C D S N I L  
871 AAGACAATAAAGATTCCTCCAAAGGAAAAACA  
TTCGTGTTATTTCTAAGGGTTTCCTTTTGT  
276 K T I K I P K G K T  
901 TGCCAACTACTTTTCCAACTCTCCAAAGCTGAAAAATTTGTCTTTTCTGGATGCTCAAGTACTCAGAGTT  
ACGGTTGGATGAAAGGTTGAGAGGTTTCGACTTTTAAACAGAAAAAGACCTACGAGTTTCATGAGTCTCAA  
286 C Q P T F Q L S K A E K F V F S G C S S T Q S Y  
971 ACAAAACCCACTTTTTGTGGAATATGCTTGG  
TGTTTGGGTGAAAAACACCTTATACGAACC  
310 K P T F C G I C L D

**FIG. 6B**

```

1001 ATAGAGATGCTGTATCCCTAATAAGTCTAAATGATTACTATTCAATTTGATTGCCCAAAATGAGGGGTC
    TATTTCTCTACGACATAGGGATTATTTCAGATTTTACTAATGATAAGTTAACTAACGGGTTTACTCCCCCAG
320  K R C C I P N K S K M I T I Q F D C P N E G S

1071 ATTTAAATGGAAGATGCTGTGGATTACATC
    TAAATTTACCTTCTACGACACCCCTAATGTAG
343  F K W K M L W I T S

1101 TTGTGTGTGCAGAGAAACTGCAGAGAACTGGAGATATATTTTCTGAGCTCAAGATTCTGTAAAAACCAA
    AACACACACAGTCTCTTTTGACGTCCTCTTGGACCTCTATATAAAGACTCGAGTTCTAAGACATTTTGGTT
353  C V C Q R N C R E P G D I F S E L K I L O

1171 GCAAATGGGGGAAAAGTTAGTCAATCCTGT
    CGTTTACCCCTTTTCAATCAGTTAGGACA

1201 CATANAATAAAAAAATTAGTGAGTATATAAATGGTGGCAAAATCTACTTTGTTTAAACAGTATGAATGCCT
    GTATNTTATTTTTTAAATCACTCATATTTTACCACCGTTTAGATGAACAACAAATTTTGTCTACTACTTACGGA

1271 ATTCCTCAGATCAGTACATTTAAGGCATTAG
    TAAGAGTCTAGTGATGTAAATTCGGTAATC

1301 AAACTTTTTAAAAAGTTTANCCTTAAAAATATACATAA
    TTTTGAAAAATTTTTCAATNGAATTTTTTATATGTATT

```

**FIG.\_6C**

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1 CACGGTCCCAGGACATGCAGGGGCTCCTCTCTCCACTCTTCTGCTTGTGGCCTGGCACAGTTCTGCT  
 GTGCCAGGGTCGCTGTACGTCCCGAGGAGAGAGGTGAGAAGACGACCGGACCGTGTCAAGACGA  
 1 M Q G L L F S T L L L A G L A Q F C C C  
 71 GCAGGGTACAGGGCACTGGACCANTAGATA  
 CGTCCCATGTCCCGTGACCTGGTAATCTAT  
 20 R V Q G T G P L D T  
 101 CAACACCTGAAGGAAGCCCTGGAGAAGTGTCAAGATGCACCTCAGCGTAAACAGTTTGTCACTGGCCCCTG  
 GTTGTGGACTTCTTCCGGACCTCTTCACAGTCTACGTGGAGTGCATTTGTCAAAACAGTGACCGGGAC  
 30 T P E G R P G E V S D A P Q R K Q F C H W P C  
 171 CAAATGCCCTCAGCAGAGAGCCCGTTGCC  
 GTTACGGGAGTCGTCTTCGGGGCAACGGG  
 53 K C P Q Q K P R C P  
 201 TCCTGGAGTGAGCCCTGGTGAGAGATGGCTGTGATGCTGTAAATCTGTCCAAAGCAACCAGGGGAATC  
 AGGACCTCACTCGGACCACTCTCTACCGACACCTACGACATTTAGACACGGTTCTGGTCCCTTTAG  
 63 P G V S L V R D G C G C K I C A K Q P G E I  
 271 TGCAATGAAGCTGACCTCTGTGACCCACAC  
 ACGTTACTTCGACTGGAGACACTGGGTGTG  
 86 C N E A D L C D P H  
 301 AAAGGGCTGTATTGTGACTACTCAGTAGACAGGCCCTAGGTACGAGACTGGAGTGTGCATACCTTTGTAG  
 TTTCCCGACATAACACTGATGATCATCTGTCCGGATCCATGCTCTGACCTCACACGTAATGGAACATC  
 96 K G L Y C D Y S V D R P R Y E T G V C A Y L V A  
 371 CTGTTGGGTGGAGTTCAACCAGGTACATT  
 GACAACCCACGCTCAAGTTGGTCCATGTAA  
 120 V G C E F N Q V H Y  
 401 ATCAATAATGGCCAAGTGTTCAGCCCCAACCCCTTGTTCAGCTGCCCTCTGTGTAGTGGGCCATTGGATG  
 TAGTATTACCGGTTCAAAAGTCGGGTGGGGAACAAGTCGACGGAGACACACTCACCCCGGTAACCTAC  
 130 H N G Q V F Q P N P L F S C L C V S G A I G C  
 471 CACACCTCTGTTTCATACCAAGCTGGCTGG  
 GTGTGGAGACAAGTATGGTTTCGACCGACC  
 153 T P L F I P K L A G

FIG. 7A

501 CAGTCACTGCTCTGGAGCTAAAGGTGGAAGAAGTCTGATCACTCAAACTGTAGCCTGGAACCACTTACTA  
 GTCAGTGACGAGACCTCGATTCCACCCTTCTTCAGACTAGTCAGTTTGACATCGGACCTTGGTAATGAT  
 163 S H C S G A K G G K K S D Q S N C S L E P L L  
 571 CAGCAGCTTTCACAAGCTACAAAACAATG  
 GTCGTCGAAAGTTGTTTCGATGTTTTGTAC  
 186 Q Q L S T S Y K T M  
 601 CCAGCTTATAGAAATCTCCCACTTATTGGAAAAAATGCTTGTGCAAGCAACAATGGACTCCCT  
 GGTGGAATATCTTTAGAGGTGAATAAACCTTTTTTTTACAGAACACGTTCTGTTGTTTACCTGAGGGA  
 196 P A Y R N L P L I W K K C L V Q A T K W T P C  
 671 GCTCCAGAACATGTGGGATGGGAATATCTA  
 CGAGGCTTGTACACCCCTACCCCTTATAGAT  
 220 S R T C G M G I S N  
 701 ACAGGGTGACCAATGAAAACAGCAACTGTGAAATGAGAAAAAGAAAGACTGTGTACATTTCAGCCTTG  
 TGTCCCACTGGTTACTTTTGTGCTTGACACTTACTCTTCTCTTTCTGACACAATGTAAGTCGGAAC  
 230 R V T N E N S N C E M R K E K R L C Y I Q P C  
 771 CGACAGCAATATATTAAAGACAATAAGAT  
 GCTGTCGTTATATAATTTCTGTTATTCTA  
 253 D S N I L K T I K I  
 801 TCCCAAAGGAAAAACATGCCAACCTACTTTCCAACTCTCCAAAGCTGAAAAATTTGTCTTTCTGGATGC  
 AGGGTTTCCTTTTGTACGGTTGGATGAAGGTTGAGAGGTTTCGACTTTTAAACAGAAAAAGACCTACG  
 263 P K G K T C Q P T F Q L S K A E K F V F S G C  
 871 TCAAGTACTCAGAGTTACAAACCCACTTTT  
 AGTTCATGAGTCTCAATGTTTGGGTGAAAA  
 286 S S T Q S Y K P T F

**FIG. 7B**



```

901 TGCGAATATGCTTGGATAAGAGATGCTGTATCCCTAATAAGTCTAAAGTGAATGATTCAATTGATT
  ACACCTTATACGAACCTATTCTCTACGACATAGGGAATTATTTCAGATTTTACTAATGATAAAGTTAAACTAA
296 C G I C L D K R C C I P N K S K M I T I Q F D C
          GCCCAAATGAGGGGTCATTTAAATGGAAGA
971 CGGGTTTACTCCCCAGTAAATTTACCTTCT
          P N E G S F K W K M
320
1001 TGCTGTGGATTACATCTTGTGTGTGTGTCAGAGAAACTGCAGAGAACCTGGAGATATATTTCTGAGCTCAA
  ACGACACCTAATGTAGAACACACACAGTCTCTTTGACGTCCTTTGGACCTCTATATATAAAGACTCGAGTT
330 L W I T S C V C Q R N C R E P G D I F S E L K
          GATTCTGTAAACCAAGCAAATGGGGAAA
1071 CTAAGACATTTTGGTTCGTTTACCCCTTT
          I L O
353
1101 AGTTAGTCAATCCTGTCAATATAATAAAAAAATTAGTGTAGTAAAAAATAAAAAAATAAAAAA
  TCAATCAGTTAGGACAGTATATTATTTTAAATCACTCATTTTTTTTTTTTTTTTTTTTTTTTTTTT
          AAAAAAAAAAAAAAAAAAAGAAAAAAA
1171 TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
          AAAAAAAAAA
1201 TTTTTTTTTTTT

```

FIG.-7C

mouse.wisp-1	1	MRWL	LPWTLAAV	AVLRVGNIL	LATALSPT	PTTMT	FTPAPLE	ETTRPE	EFCK
human.wisp-1	1	MRWF	LPWTLAAV	TAAASTV	LATALSPT	PTTMD	FTPAPLE	DTSSRP	QFCK
mouse.wisp-1	51	WPCECP	QSPPRCPL	GVSLIT	DGCECK	I	CAQQLG	DNCTEAA	ICDPHRGLY
human.wisp-1	51	WPCECP	PSPPRCPL	GVSLIT	DGCECK	M	CAQQLG	DNCTEAA	ICDPHRGLY
mouse.wisp-1	101	CDYSGDR	PRYAIGV	CAQVVG	VCVLDG	VRYT	NGES	FQPNCR	YNCTCIDGT
human.wisp-1	101	CDYSGDR	PRYAIGV	CAQVVG	VCVLDG	VRYN	NGQS	FQPNCK	YNCTCIDGA
mouse.wisp-1	151	VGCTPL	CLSP	RPRLWC	RQPRH	VRVP	QGQ	CCCEQWVC	DDARRPRQT
human.wisp-1	151	VGCTPL	CLRV	RPRLWC	PHPRV	SI	PGH	CCCEQWVC	EDDAKRRPRKT
mouse.wisp-1	201	RAFA	ASGAVE	QRYENC	IAYTSP	WSPCST	TCGLG	I	STRISNVNAR
human.wisp-1	201	GAFA	DVGEVE	AWHRNC	IAYTSP	WSPCST	SCGLG	V	STRISNVNAQ
mouse.wisp-1	251	SRLCNL	RPCDVDI	QLH	IKAGKK	CLAVYQPE	EAT	NFTLAGC	VSTRIT
human.wisp-1	251	SRLCNL	RPCDVDI	HTL	IKAGKK	CLAVYQPE	ASMN	NFTLAGC	IISTRISYQPKY
mouse.wisp-1	301	CGVC	T	DNRC	CI	PKSKTI	SV	DFQCP	EGP
human.wisp-1	301	CGVC	M	DNRC	CI	PKSKTI	DVS	DFQCP	DGL
mouse.wisp-1	351	DIFADLES	YPDFE	EIAN					
human.wisp-1	351	DIFADLES	YPDFS	EIAN					

FIG.\_8

mouse.wisp-2	1	M	R	G	N	P	L	I	H	L	L	A	I	S	F	L	C	I	L	S	M	V	Y	S	Q	L	C	P	A	P	C	A	C	P	W	T	P	P	Q	C	P	P	G	V	P	L	V	L	D	G	C
human.wisp-2	1	M	R	G	T	P	K	T	H	L	L	A	F	S	L	L	C	L	L	S	K	V	R	T	Q	L	C	P	T	P	C	T	C	P	W	P	P	P	R	C	P	L	G	V	P	L	V	L	D	G	C
mouse.wisp-2	51	G	C	C	R	V	C	A	R	R	L	G	E	S	C	D	H	L	H	V	C	D	P	S	Q	G	L	V	C	P	G	A	G	P	S	G	R	G	A	V	C	L	F	E	E	D	D	G	S	C	
human.wisp-2	51	G	C	C	R	V	C	A	R	R	L	G	E	P	C	D	Q	L	H	V	C	D	A	S	Q	G	L	V	C	P	G	A	G	P	G	G	R	G	A	L	C	L	A	E	D	D	S	S	C		
mouse.wisp-2	101	E	V	N	G	R	R	Y	L	D	G	E	T	F	K	P	N	C	R	V	L	C	R	C	D	D	G	G	F	T	C	L	P	L	C	S	E	D	V	R	L	P	S	W	D	C	P	R	P	R	R
human.wisp-2	101	E	V	N	G	R	L	Y	R	E	G	E	T	F	Q	P	H	C	S	I	R	C	R	C	E	D	G	G	F	T	C	V	P	L	C	S	E	D	V	R	L	P	S	W	D	C	P	H	P	R	R
mouse.wisp-2	151	I	Q	V	P	G	R	C	C	P	E	W	V	C	D	Q	A	V	M	Q	P	A	I	Q	P	S	S	A	Q	G	H	Q	L	S	A	L	V	T	P	A	S	A	D	G	P	C	P	N	W	S	T
human.wisp-2	151	V	E	V	L	G	K	C	C	P	E	W	V	C	G	Q	G	G	-	G	L	G	T	Q	P	L	P	A	Q	G	P	Q	F	S	G	L	V	S	S	L	P	P	G	V	P	C	P	E	W	S	T
mouse.wisp-2	201	A	W	G	P	C	S	T	T	C	G	L	G	I	A	T	R	V	S	N	Q	N	R	F	C	Q	L	E	I	Q	R	R	L	C	L	S	R	P	C	L	A	S	R	S	H	G	S	W	N	S	A
human.wisp-2	200	A	W	G	P	C	S	T	T	C	G	L	G	M	A	T	R	V	S	N	Q	N	R	F	C	R	L	E	T	Q	R	R	L	C	L	S	R	P	C	P	P	S	R	G	R	S	P	Q	N	S	A
mouse.wisp-2	251	F																																																	
human.wisp-2	250	F																																																	

FIG.\_9

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	10	20	30	40	50
hWISP-3.DNA56350	MNKRRLLYPSGWLHGP	SDMQGLLFSTLLLAGLAQFCCRVQGTG	PLD	TTPE	
	*****				
hWISP-3.DNA58800		MQGLLFSTLLLAGLAQFCCRVQGTG	PLD	TTPE	
	10	20	30		
	60	70	80	90	100
hWISP-3.DNA56350	GRPGEVSDAPQRKQFCHWPCKCPQ	QKPRCPPGVSLVRDGC	GCKKICAKQ		
	*****				
hWISP-3.DNA58800	GRPGEVSDAPQRKQFCHWPCKCPQ	QKPRCPPGVSLVRDGC	GCKKICAKQ		
	40	50	60	70	80
	110	120	130	140	150
hWISP-3.DNA56350	GEICNEADLCDPHKGLYCDYSVDR	PRYETGVCAYLVAVGCE	FNQVHYHNG		
	*****				
hWISP-3.DNA58800	GEICNEADLCDPHKGLYCDYSVDR	PRYETGVCAYLVAVGCE	FNQVHYHNG		
	90	100	110	120	130
	160	170	180	190	200
hWISP-3.DNA56350	QVFQPNPLFSCLCVSGAIGCTPLF	FIPKLAGSHCSGAKGGKSDQ	SNC	SLE	
	*****				
hWISP-3.DNA58800	QVFQPNPLFSCLCVSGAIGCTPLF	FIPKLAGSHCSGAKGGKSDQ	SNC	SLE	
	140	150	160	170	180
	210	220	230	240	250
hWISP-3.DNA56350	PLLQQLSTSYKTMPAYRDLPLI	WKKKCLVQATKWTPCS	RTCGMGISNRVT		
	*****				
hWISP-3.DNA58800	PLLQQLSTSYKTMPAYRNLPLI	WKKKCLVQATKWTPCS	RTCGMGISNRVT		
	190	200	210	220	230
	260	270	280	290	300
hWISP-3.DNA56350	NENSNCEMRKEKRLCYIQPCDS	NIKTIKIPKGKTCQPTF	QLSKA	EKFVF	
	*****				
hWISP-3.DNA58800	NENSNCEMRKEKRLCYIQPCDS	NIKTIKIPKGKTCQPTF	QLSKA	EKFVF	
	240	250	260	270	280
	310	320	330	340	350
hWISP-3.DNA56350	SGCSSTQSYKPTFCGICLDKRCC	IPNKS	KMITIQFDCPNEGS	FKWKMLWI	
	*****				
hWISP-3.DNA58800	SGCSSTQSYKPTFCGICLDKRCC	IPNKS	KMITIQFDCPNEGS	FKWKMLWI	
	290	300	310	320	330
	360	370			
hWISP-3.DNA56350	TSCVCQRNCREPGDIFSELKIL				
	*****				
hWISP-3.DNA58800	TSCVCQRNCREPGDIFSELKIL				
	340	350			

**FIG. 10**

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hWISP-3.DNA56350  GTGGGGTTTGCAGAGGAGACAGGGGAGCTTTGTGTACCCGGAGCAATGAA
                      10      20      30      40      50

huWISP-1
                      A
                      *

hWISP-3.DNA56350  CAAGCGGCGACTTCTCTACCCCTCAGGGTGGCTCCACGGTCCCAGCGACA
                      60      70      80      90     100

                      10      20      30      40
huWISP-1          TGAGGTGGTTCTTGCCTTGGAC---GCTGGCAGCAGTGACAGCAGCAGCC
** * * * * *      ** **      *** ** *      *** *
hWISP-3.DNA56350  TGCAGGGGCTCCTCTTCTCCACTCTTCTGCTTGCTGGCCTGGCACAGTTC
                      110     120     130     140     150

                      50      60      70      80      90
huWISP-1          GCCAGCACCGTCCTGGCCACGGCCCTCTCTCCAGCCCCTACGACCATGGA
* * * * *      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  TGCTGCAGGGTACAGGGCACTG-----GACCATTAGATACAACACCTGA
                      160     170     180     190

                      100     110     120     130     140
huWISP-1          CTTTACTCCAGCTCCACTGGAGGACACCTCCTCACGCCCCCAATTCTGCA
* * * * *      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  AGGAAGGCCTGGAGAAGTGTGAGATGCACCTCAGCGTAAACAGTTTGTGTC
                      200     210     220     230     240

                      150     160     170     180     190
huWISP-1          AGTGGCCATGTGAGTGCCCGCCATCCCCACCCCGCTGCCCCGCTGGGGGTC
* * * * *      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  ACTGGCCCTGCAAATGCCCTCAGCAGAAGCCCCGTTGCCCTCCTGGAGTG
                      250     260     270     280     290

                      200     210     220     230     240
huWISP-1          AGCCTCATCACAGATGGCTGTGAGTGCTGTAAGATGTGCGCTCAGCAGCT
*****      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  AGCCTGGTGAGAGATGGCTGTGGATGCTGTAAAATCTGTGCCAAGCAACC
                      300     310     320     330     340

                      250     260     270     280     290
huWISP-1          TGGGGACAACATGCACGGAGGCTGCCATCTGTGACCCCCACCGGGGCCTCT
*****      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  AGGGGAAATCTGCAATGAAGCTGACCTCTGTGACCCACACAAAGGGCTGT
                      350     360     370     380     390

                      300     310     320     330     340
huWISP-1          ACTGTGACTACAGCGGGGACCGCCCGAGGTACGCAATAGGAGTGTGTGCA
* * * * *      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  ATTGTGACTACTCAGTAGACAGGCCTAGGTACGAGACTGGAGTGTGTGCA
                      400     410     420     430     440

                      350     360     370     380     390
huWISP-1          CAGGTGGTTCGGTGTGGGCTGCGTCTGGATGGGGTGCGCTACAACAACGG
* * * * *      * * * * *      * * * * *      * * * * *
hWISP-3.DNA56350  TACCTTGTAGCTGTTGGGTGCGAGTTCAACCAGGTACATTATCATAATGG
                      450     460     470     480     490

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**FIG. 11A**

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	400	410	420	430	440
huWISP-1	CCAGTCCTTCCAGCCTAACTGCAAGTACAAGTGCACGTGCATCGACGGCG				
	*** ** * * * * * * * * * * * * * * * * * *				
hWISP-3.DNA56350	CCAAGTGTTTCAGCCCAACCCCTTGTTTCAGCTGCCTCTGTGTGAGTGGGG				
	500	510	520	530	540
	450	460	470	480	490
huWISP-1	CGGTGGGCTGCACACCACTGTGCCTCCGAGTGCGCCCGCGCTCTCTGG				
	* *				
hWISP-3.DNA56350	CCATTGGATGCACACCTCTGTTTCATACCAAAGC-----TGGCTGG				
	550	560	570		580
	500	510	520	530	540
huWISP-1	TGCCCCCACC CGCGCGCTGAGC-ATACCTGGCCACTGCTGTGAGCAGT				
	** * * * * * * * * * * * * * * * * * *				
hWISP-3.DNA56350	-----CAGTCACTGCTCTGGAGCTAAAGGTGGAAAGAAGTCTGATCAGT				
	590	600	610	620	
	550	560	570	580	590
huWISP-1	GGGTATGTGAGGACGACGCCAAGAGGCCACGCAAGACCGCACCCCGTGAC				
	*** ** * * * * * * * * * * * * * * * *				
hWISP-3.DNA56350	CAAAGTGT-AGCCTGGAACCATTA--CTACAGCAGCTTTCACAAGCTAC				
	630	640	650	660	670
	600	610	620	630	640
huWISP-1	ACAGGAGCCTTCGATGCTGTGGGTGAGGTGGAGGCATGGCACAGGAAGT				
	* *				
hWISP-3.DNA56350	AAAACAATGCCAGCTTATAGAGATCTCCCACTTATTTGGAAAAAAAATG				
	680	690	700	710	720
	650	660	670	680	690
huWISP-1	CATAGCCTACACAAGCCCCCTGGAGCCCTTGCTCCACCAGCTGCGGCCTGG				
	* *				
hWISP-3.DNA56350	TCTTGTGCAAGCAACAAAATGGACTCCCTGCTCCAGAACATGTGGGATGG				
	730	740	750	760	770
	700	710	720	730	740
huWISP-1	GGGTCTCCACTCGGATCTCCAATGTTAACGCCAGTGCTGGCCTGAGCAA				
	* *				
hWISP-3.DNA56350	GAATATCTAACAGGGTGACCAATGAAAACAGCAACTGTGAAATGAGAAAA				
	780	790	800	810	820
	750	760	770	780	790
huWISP-1	GAGAGCCGCTCTGCAACTTGCGGCCATGCGATGTGGACATCCATACACT				
	**** * * * * * * * * * * * * * * * *				
hWISP-3.DNA56350	GAGAAAAGACTGTGTTACATTCAGCCTTGCGACAGCAATATATTAAAGAC				
	830	840	850	860	870
	800	810	820	830	840
huWISP-1	CATTAAG-----GCAGGGAAGAGTGTCTGGCTGTGTACCAGCCAGAGG				
	** *** * * * * * * * * * * * * * * * *				
hWISP-3.DNA56350	AATAAAGATTCCCAAAGGAAAAACATGCCAACCTACTTTCCAAGTCTCCA				
	880	890	900	910	920
	850	860	870	880	890
huWISP-1	CATCCATGAAGTTACACTTGCGGGCTGCATCAGCACACGCTCCTATCAA				
	* *				
hWISP-3.DNA56350	AAGCTGAAAAATTTGTCTTTTCTGGATGCTCAAGTACTCAGAGTTACAAA				
	930	940	950	960	970

**FIG. 11B**

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	900	910	920	930	940
huWISP-1	CCCAAGTACTGTGGAGTTTGCATGGACAATAGGTGCTGCATCCCCCTACAA				
	**** * ***** * *** ***** ** * ***** ***** * **				
hWISP-3.DNA56350	CCCACCTTTTGTGGAATATGCTTGGATAAGAGATGCTGTATCCCTAATAA				
	980	990	1000	1010	1020
	950	960	970	980	990
huWISP-1	GTCTAAGACTATCGACGTGTCCTTCCAGTGTCTGATGGGCTTGGCTTCT				
	***** * ** * ** * ** * ** * ** *				
hWISP-3.DNA56350	GTCTAAAATGATTACTATTCAATTTGATTGCCCAAATGAGGGGTCATTTA				
	1030	1040	1050	1060	1070
	1000	1010	1020	1030	1040
huWISP-1	CCCGCCAGGTCCTATGGATTAATGCCTGCTTCTGTAACCTGAGCTGTAGG				
	* * * * * ***** * ** * ** * * * * * *				
hWISP-3.DNA56350	AATGGAAGATGCTGTGGATTACATCTTGTGTGTGTGCAGAGAACTGCAGA				
	1080	1090	1100	1110	1120
	1050	1060	1070	1080	1090
huWISP-1	AATCCCAATGACATCTTTGCTGACTTGGAATCCTACCCTGACTTCTCAGA				
	* ** ** * * * * * * * * * ** * * *				
hWISP-3.DNA56350	GAACCTGGAGATATATTTTCTGAGCTCAAGATTCTGTAAAACCAAGCAAA				
	1130	1140	1150	1160	1170
	1100				
huWISP-1	AATTGCCAAC				
	* **				
hWISP-3.DNA56350	TGGGGGAAAAGTTAGTCAATCCTGTCATANAATAAAAAAATTAGTGAGTA				
	1180	1190	1200	1210	1220
hWISP-3.DNA56350	TAAAATGGTGGCAAATCTACTTTGTTTAAAACAGTATGAATGCCTATTCT				
	1230	1240	1250	1260	1270
hWISP-3.DNA56350	CAGATCACTACATTTAAGGCATTAGAACTTTTAAAAAGTTANCTTAAAA				
	1280	1290	1300	1310	1320
hWISP-3.DNA56350	ATATACATAA				
	1330				

**FIG. 11C**

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	10	20	30	40
hWISP-3.DNA56350	MNKRRLLYPSGWLHG	PSDMQGLLFSTL-LLAGLAQFCCR	VQGTGPLD	TTP
		*. * ** ... *		* **
huWISP-1		MRWFLPWTLAAVTA	AAASTVLATALSP	APTTM
		10	20	30
	50	60	70	80
hWISP-3.DNA56350	EGRPGEVSDAPQRKQFCHWPCKCPQ	QKPRCP	PGVSLVRDGC	CGCKICAKQ
	. * . * . * * * * * * * * * * * * * * * *			
huWISP-1	DFTPAPLED	TSSRPQFCKWPCECPPSP	PRCPLGVSLITD	GCECKMCAQQ
	40	50	60	70
	100	110	120	130
hWISP-3.DNA56350	PGEICNEADL	CDPHKGLYCDYSVDRPRY	ETGVCAYLVAVGCE	FNQVHYHN
	*. * ** . * * * . * * * * * * * * * * . * . * . *			
huWISP-1	LGDNCTEAAICDP	HRGLYCDYSGDRPRYAIGVCAQ	VVGVCVLDG	VRYNN
	90	100	110	120
	150	160	170	180
hWISP-3.DNA56350	GQVFQPNPLF	SCLCVSGAIGCTPL-FIPKLAGSH	CSGAK----	GGKKS
	** * * * . * * . * * . * * * * . * . . . *			
huWISP-1	GQSFQPNCKYNCT	CIDGAVGCTPLCLVRP	PPRLWCPHPR	VSI
	140	150	160	170
	200	210	220	230
hWISP-3.DNA56350	SNCSLEPLLQQL	STSYKTM	PAYRDLPLI--WKKKCLVQAT	KWT
	* . . . . . * . . . * . . . * * * * . *			
huWISP-1	WVCEDDAKRPR	KTAP-RDTGAFDAVGEVEA	WHRNCIAYTSPW	SPCSTSCG
	190	200	210	220
	250	260	270	280
hWISP-3.DNA56350	MGISNRVTNENS	NCNCEMRKEKRLCYIQPCDSN	ILKTIKIPK	GKTCQPTFQL
	. * . * . * . * . * . * * * * . * * * * . *			
huWISP-1	LGVSTRISNVNA	QCWPEQESRLCNLRPCDV	DIHTLIK--AGKKCLAVY	Q
	240	250	260	270
	300	310	320	330
hWISP-3.DNA56350	SKAEKFV	FGCSSTQSYKPTFCGICLDK	RCCIPNKS	KMITIQFDCPNEGS
	. * . * . * * * . * * * . * * * * * * * * . * . *			
huWISP-1	EASMNFTLAG	CISTR	SYQPKYCGVCM	DNRC
	280	290	300	310
	350	360	370	
hWISP-3.DNA56350	FKWKMLWIT	SCVCQRNCREPGDIF	SELKIL	
	* . . * * * . * * . * * * * * * * *			
huWISP-1	FSRQVLWINAC	FCNLSCRNPNDIFADLESYP	PDFSEIAN	
	330	340	350	360

FIG. 12



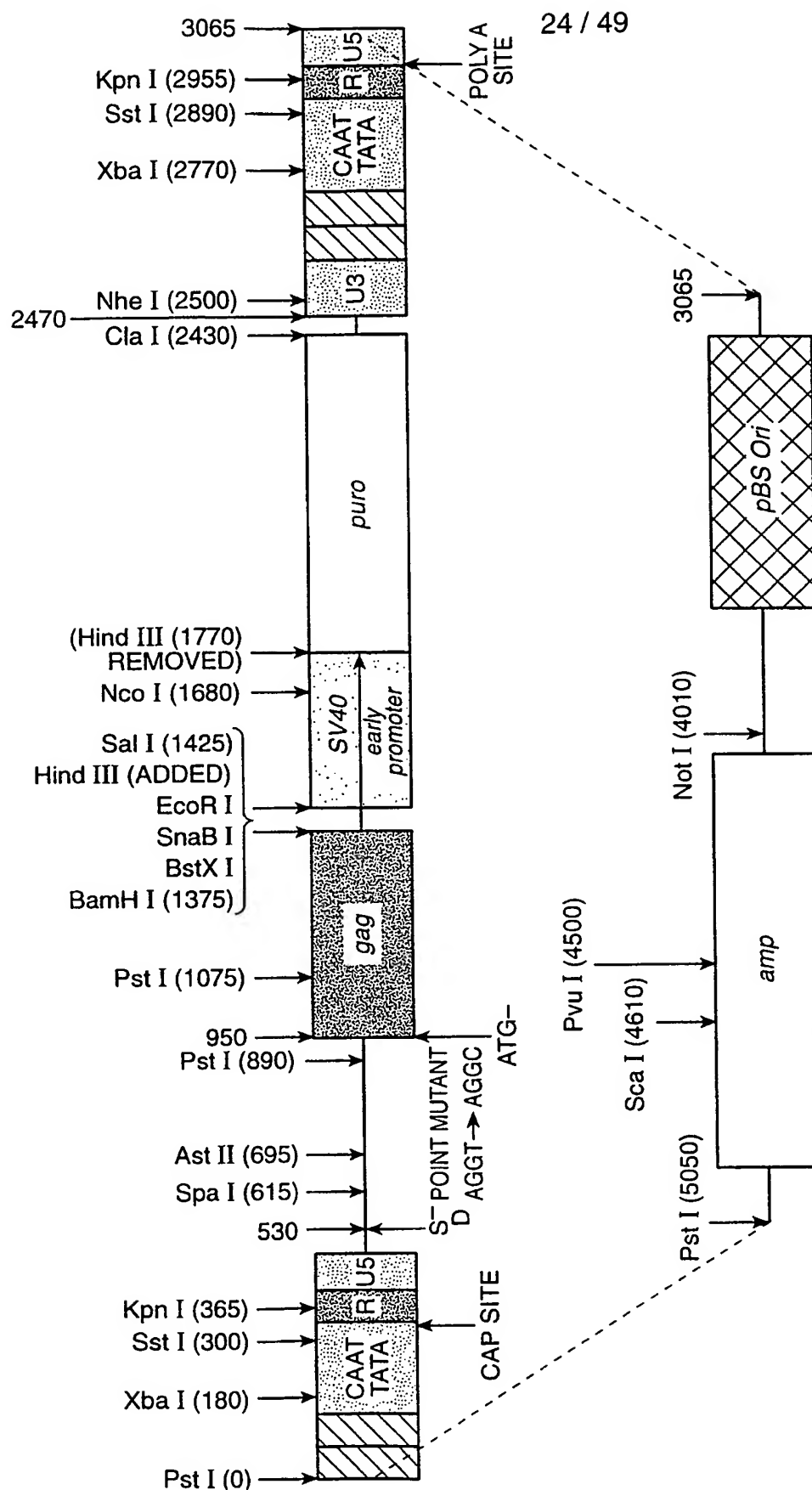
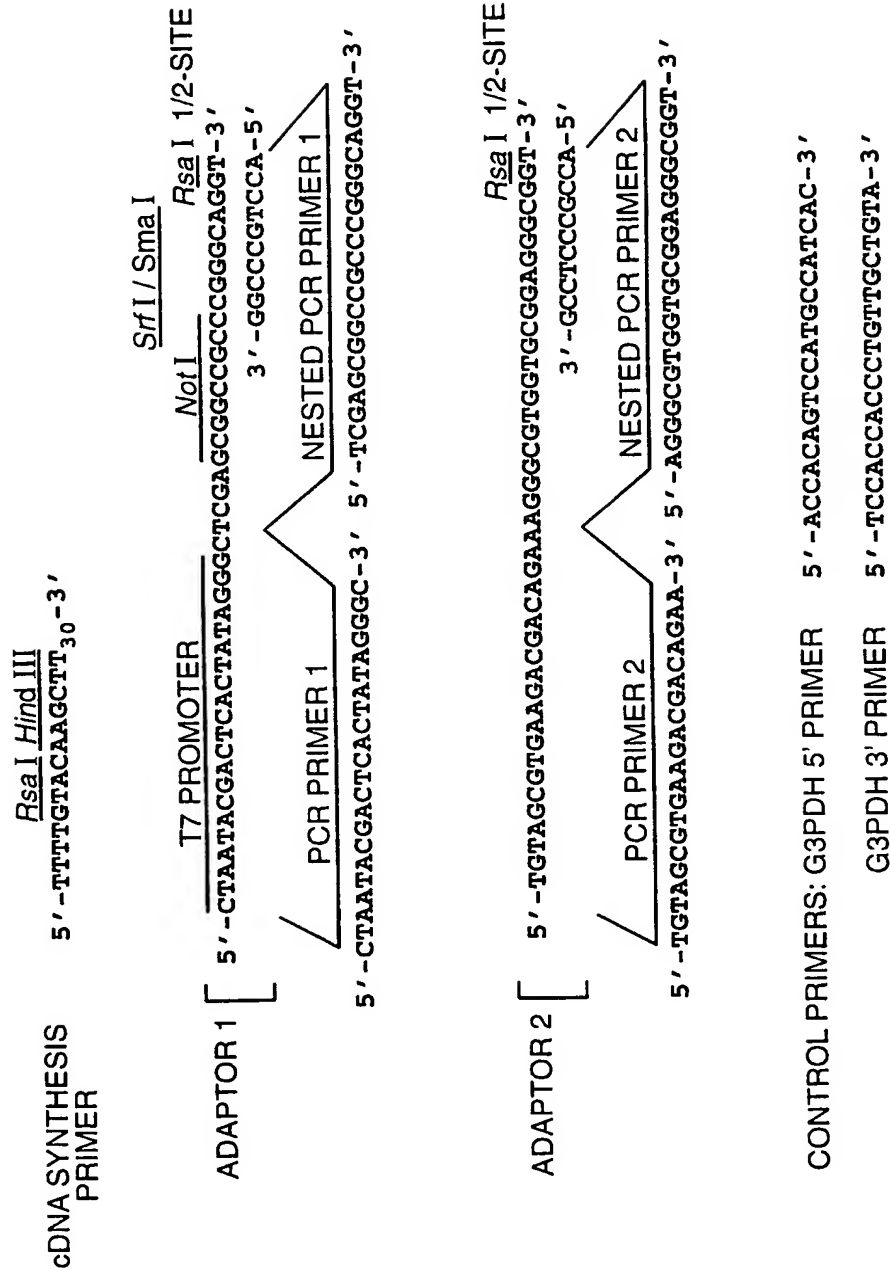


FIG. 13



**FIG. 14**

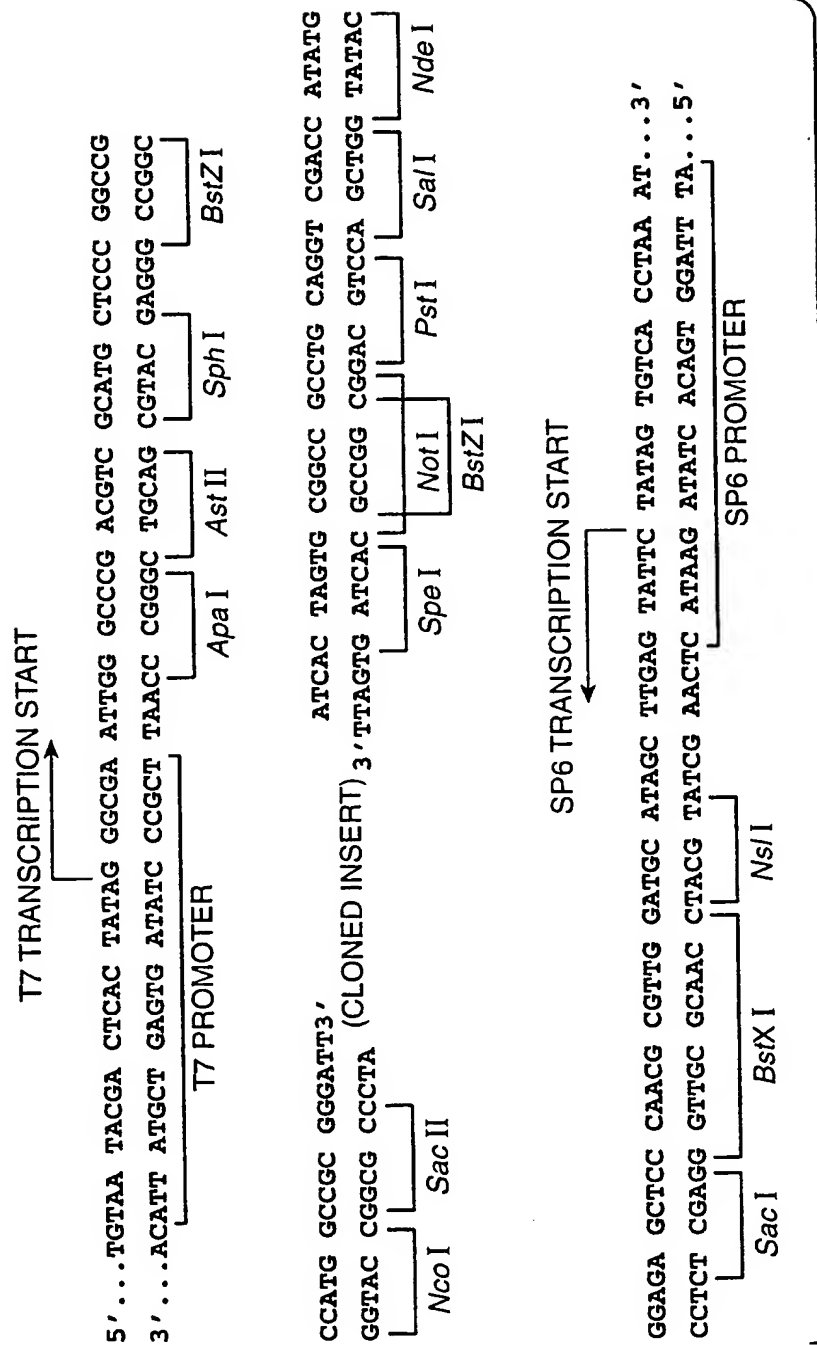


FIG. 15

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TTCGAGCTCGCCCGACATTGATTATTGACTAGAGTCGATCACCGGTTATTAATAGTAATC  
AATTACGGGGTCATAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTA  
AATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTAT  
GTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG  
TAAACTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC  
GTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTTATGGGACTTT  
CCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGG  
CAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCC  
ATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT  
AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATA  
AGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGAC  
CTGGGCCCCGCGCCGCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTT  
TTTGGAGGCCCTAGGCTTTTGGCAAAAAGCTAGCTTATCCGGCCGGGAACGGTGCAATTGGAA  
CGCGGATTCCCCGTGCCAAGAGT  
><splice donor>  
GACGTAAGTACCGCCTATAGAGCGACTAGTCCACC  
><PUR>  
ATGACCGAGTACAAGCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCGCGGGCCGTA  
CGCACCTCGCCGCGCGGTTTCGCCGACTACCCCGCCACGCGCCACACCGTAGACCCGGAC  
CGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCGCGTCGGGCTCGAC  
ATCGGCAAGGTGTGGGTTCGCGGACGACGGCGCCGCGGTGGCGGTCTGGACCACGCCGGAG  
AGCGTCGAAGCGGGGGCGGTGTTCGCCGAGATCGGCCCCGCGCATGGCCGAGTTGAGCGGT  
TCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTGGCGCCGCACCGGCCCAAGGAG  
CCCGCGTGGTTCTGGCCACCGTCGGCGTCTCGCCCCGACCACCAGGGCAAGGGTCTGGGC  
AGCGCCGTCGTGCTCCCCGGAGTGGAGGCGGCGAGCGCGCCGGGGTGCCCGCCTTCCTG  
GAGACCTCCGCGCCCCGCAACCTCCCTTCTACGAGCGGCTCGGCTTCACCGTCACCGCC  
GACGTCGAGTGCCCGAAGGACCGCGCGACCTGGTGCATGACCCGCAAGCCCGGTGCCAAC  
><DHFR ATG>  
ATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATTGGCAAGAAC  
GGAGACCTACCCTGCCCTCCGCTCAGGAACGCGTTCAAGTACTTCCAAAGAATGACCACA  
ACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCTCC  
ATTCTTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAACTC  
AAAGAACCACCGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGACTT  
ATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGTTCT  
GTTTACCAGGAAGCCATGAATCAACCAGGCCACCTTAGACTCTTTGTGACAAGGATCATG  
CAGGAATTTGAAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAACCTCTC  
CCAGAATACCCAGGCGTCTCTCTGAGGTCCAGGAGGAAAAGGCATCAAGTATAAGTTT  
GAAGTCTACGAGAAGAAAGACTAA  
><End DHFR>  
CGTTAACTGCTCCCCTCCTAAAGCTATGCATTTTTTATAAGACCATGGGACTTTTGCTGGC  
TTTAGATCCCCCTTGGCTTCGTTAGAACGCAGCTACAATTAATACATAACCTTATGTATCA  
TACACATACGATTTAGGTGACACTATAGATAACATCCACTTTGCCTTTCTCTCCACAGGT  
GTCCACTCCCAGGTCCAACCTGCACCTCGGTTCTATCGATTGAATTCCCCGGGGATCCTCT  
AGAGTCGACCTGCAGAAGCTTCGATGGCCGCCATGGCCCAACTTGTTTATTGCAGCTTAT  
AATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTG  
CATCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCGATCGG  
<sv40 origin>  
GAATTAATTCCGGCGCAGCACCATGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTA  
<Kpn-SAR-Kpn insert here>  
GGTACCGACTAGTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA

**FIG. 16A**

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CGACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGAC  
TTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCA  
AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTG  
GCATTATGCCCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCAATGGGCGTGGATAGCG  
AGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGAGTTTGTTTTG  
GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTG  
ACTAGTAGCAAGGTCGCCACGCACAAGATCAATATTAAACAATCAGTCATCTCTCTTTAGC  
AATAAAAAGGTGAAAAATTACATTTTAAAAATGACACCATAGACGATGTATGAAAATAAT  
CTACTTGGAAATAAATCTAGGCAAAGAAGTGCAAGACTGTTACCCAGAAAACCTTACAAAT  
TGTAATGAGAGGTTAGTGAAGATTTAAATGAATGAAGATCTAAATAAACTTATAAATTG  
TGAGAGAAATTAATGAATGTCTAAGTTAATGCAGAAACGGAGAGACATACTATATTCATG  
AAGTAAAGACTTAATATTGTGAAGGTATACTTTCTTTTCACATAAATTTGTAGTCAATA  
TGTTCACCCCAAAAAGCTGTTTGTAACTTGTCAACCTCATTTCAAAATGTATATAGAA  
AGCCCAAAGACAATAACAAAAATATTCTTGTAGAACAATAATGGGAAAGAATGTTCCACTA  
AATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGCTGATAAAA  
TAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATG  
GCATTTTACAATGGGAAAATGATGATCTTTTTCTTTTTTAGAAAAACAGGGAAATATATT  
TATATGTAAAAAATAAAAGGGAACCCATATGTCATACCATACACAAAAAATTTCCAGT  
GAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTTAGAAAAATAATATAGAAG  
CATGCCATCATGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCCTAACCACAA  
AGAAAAGATTGTTAATTAGATTGCATGAATATTAAAGACTTATTTTTTAAATTA AAAAACC  
ATTAAGAAAAGTCAGGCCATAGAATGACAGAAAAATATTTGCAACACCCCAAGTAAAGAGAA  
TTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAACTAGACAA  
AAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTC  
AGAAATCAGAGAACTATCATTTGCATATACACTAAATTAGAGAAATATTAAAAGGCTAAGT  
AACATCTGTGGCAATATTGATGGTATATAACCTTGATATGATGTGATGAGAACAGTACTT  
TACCCCATGGGCTTCTCTCCCAAAACCTTACCCCAAGTATAAATCATGACAAATATACTTT  
AAAAACCATTACCCTATATCTAACCAGTACTCCTCAAACTGTCAAGGTCATCAAAAATA  
AGAAAAGTCTGAGGAAGTGTCAAACTAAGAGGAACCCCAAGGAGACATGAGAATTATATG  
TAATGTGGCATTTCTGAATGAGATCCCGAAGCAGAAAAAGAACAGTAGCTAAAAAATAAT  
GAAATATAAATAAAGTTTGAACCTTTAGTTTTTTTTTAAAAAAGAGTAGCATTAACACGGCA  
AAGTCATTTTCATATTTTTCTTGAACATTAAGTACAAGTCTATAATTA AAAAATTTTTTAA  
ATGTAGTCTGGAACATTGCCAGAAACAGAAGTACAGCAGCTATCTGTGCTGTGCGCTAAC  
TATCCATAGCTGATTGGTCTAAAATGAGATACATCAACGCTCCTCCATGTTTTTTGT  
CTTTTTTAAATGAAAACTTTATTTTTTAAAGAGGAGTTTCAGGTTTCATAGCAAAATTGAGA  
GGAAGGTACATTCAGCTGAGGAAGTTTTCTCTATTCTAGTTTACTGAGAGATTGCAT  
CATGAATGGGTGTTAAATTTTGTCAAATGCTTTTTCTGTGTCTATCAATATGACCATGTG  
ATTTTCTTCTTTAACCTGTTGATGGGACAAATTACGTTAATGATTTTCAAACGTTGAAC  
CACCCTTACATATCTGGAATAAATCTACTTGGTGTGGTGTATATTTTTTGATACATTC  
TTGGATTCTTTTTGCTAATATTTTGTGAAAATGTTTGTATCTTTGTTCATGAGAGATAT  
TGGTCTGTGTTTTCTTTCTTGTAAATGTCATTTTCTAGTTCCGGTATTAAGGTAATGCT  
GGCCTAGTTGAATGATTTAGGAAGTATTCCTCTGCTTCTGTCTTCTGAGGTACCGCGG  
CGCCCGTCGTTTTAC

**FIG. 16B**

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<start pUC118>  
<linearization linker inserted into HpaI site>  
AACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTTAATCGCCTTGCAGCACATCCCC  
CTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGC  
GCAGCCTGAATGGCGAATGGC  
<start M13>  
GCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTTCACACCGCATACGTCAAA  
GCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCG  
CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTC  
CTTCTCGCCACGTTTCGCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGG  
GTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTGGGTGATGGTTC  
ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTT  
CTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGGCTATTC  
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGCTATTGGTTAAAAAATGAGCTGATTTA  
ACAAAAATTAAACGCGAATTTTAAACAAAATATTAACGTTTACAATTTTATGGTGCACCTCT  
CAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCCGC  
TGACGCGCCCTGACGGGCTTGCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGT  
CTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAG  
< Hinc II (2271) to GTCATC>  
< Pst I (1973) to CTGCTG>  
< Acc I (183) delete 6 bp>  
<Arbitrarily change EcoRI (1) to GAATAC>  
<pUCx 83.11.25 sequence not fully known>  
ACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTC  
TTAGACGTCAGGTGGCACTTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT  
CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATA  
ATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTT  
TGCGGCATTTTGCTTCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGC  
TGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGAT  
CCTTGAGAGTTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAAAGTTCTGCT  
ATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACA  
CTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGG  
CATGACAGTAAGAGAATTATGCAGTGTCTGCCATAACCATGAGTGATAAACTGCGGCCAA  
CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGG  
GGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGA  
CGAGCGTGACACCAGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGG  
CGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGT  
TGCAGGACCCTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGG  
AGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTC  
CCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA  
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTC  
ATATATACTTTAGATTGATTTAAAACCTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGAT  
CCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTC  
AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTG  
CTGCTTGCAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCT  
ACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTC  
TCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCT  
CGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCCG  
GTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGGTTC

**FIG. 16C**

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GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA  
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGG  
CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTA  
TAGTCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG  
GGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCTCGCCTTTTG  
CTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTAT  
TACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTC  
AGTGAGCGAGGAAGCG

<Sap-SAR-Sap insert here>

GAAGAGCCCGCGGGCAAGGTCGCCACGCACAAGATCAATATTAACAATCAGTCATCTCTC  
TTTAGCAATAAAAAGGTGAAAAATTACATTTTAAAAATGACACCATAGACGATGTATGAA  
AATAATCTACTTGGAAATAAATCTAGGCAAAGAAGTGCAAGACTGTTACCCAGAAAACCTT  
ACAAATTGTAAATGAGAGGTTAGTGAAGATTTAAATGAATGAAGATCTAAATAAACTTAT  
AAATTGTGAGAGAAATTAATGAATGTCTAAGTTAATGCAGAAACGGAGAGACATACTATA  
TTCATGAACATAAAGACTTAATATTGTGAAGGTATACCTTTCTTTTCACATAAATTTGTAG  
TCAATATGTTTACCCCCAAAAAGCTGTTTGTAACTTGTCAACCTCATTTCAAAATGTAT  
ATAGAAAGCCCCAAAGACAATAACAAAAATATTCTTGTAGAACAATAATGGGAAAGAATGTT  
CCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTGGGGATAGACAGTGAGGCTG  
ATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAA  
AATATGGCATTTTACAATGGGAAAATGATGATCTTTTTCTTTTTTAGAAAAACAGGGAAA  
TATATTTATATGTAAAAAATAAAGGGAACCCATATGTCATACCATACACAAAAAAAT  
TCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTTAGAAAAATAATA  
TAGAAGCATGCCATCATGACTTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCTTAA  
CCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAATTA  
AAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAAATTTGCAACACCCCAGTAA  
AGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAGTAAAAAATAAAACT  
AGACAAAAATTTGAACAGATGAAAGAGAAACTCTAAATAATCATTACACATGAGAACTC  
AATCTCAGAAATCAGAGAACTATCATTTGCATATACACTAAATTAGAGAAATATTAAGG  
CTAAGTAACATCTGTGGCAATATTGATGGTATATAACCTTGATATGATGTGATGAGAACA  
GTACTTTACCCCATGGGCTTCCCTCCCCAAACCCCTTACCCAGTATAAATCATGACAAATA  
TACTTTTAAAAAACCATTTACCCCTATATCTAACCAGTACTCCTCAAACTGTCAAGGTCATCA  
AAAATAAGAAAAGTCTGAGGAAGTGTCAAACTAAGAGGAACCCAAGGAGACATGAGAAT  
TATATGTAATGTGGCATTCTGAATGAGATCCCAGAACAGAAAAAGAACAGTAGCTAAAAA  
ACTAATGAAATATAAATAAAGTTTGAACCTTTAGTTTTTTTTTAAAAAAGAGTAGCATTAAC  
ACGGCAAAGTCATTTTCATATTTTTCTTGAACATTAAGTACAAGTCTATAATTAATAAATT  
TTTTAAATGTAGTCTGGAACATTGCCAGAAACAGAAGTACAGCAGCTATCTGTGCTGTGCG  
CCTAACTATCCATAGCTGATTGGTCTAAAATGAGATACATCAACGCTCCTCCATGTTTTT  
TGTTTTCTTTTTTAAATGAAAACTTTATTTTTTAAAGAGGAGTTTCAGGTTTCATAGCAAAA  
TTGAGAGGAAGGTACATTCAAGCTGAGGAAGTTTTCTCTATTCTAGTTTACTGAGAGA  
TTGCATCATGAATGGGTGTTAAATTTTGTCAAATGCTTTTTCTGTGTCTATCAATATGAC  
CATGTGATTTTTCTTCTTTAACCTGTTGATGGGACAAATTACGTTAATTGATTTTCAAACG  
TTGAACCACCCCTTACATATCTGGAATAAATTCTACTTGGTGTGGTGTATATTTTTTGAT  
ACATTCTTGGATTCTTTTTGCTAATATTTTGTGAAAATGTTTGTATCTTTGTTTCATGAG  
AGATATTGGTCTGTTGTTTTCTTTCTTGTAAATGTCATTTTCTAGTTCCGGTATTAAGGT  
AATGCTGGCCTAGTTGAATGATTTAGGAAGTATTCCCTCTGCTTCTGTCTTCTGAAGCGG  
AAGAGC

<end M13>

GCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCAGC  
ACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCA  
CTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTG  
TGAGCGGATAACAATTTACACAGGAAACAGCTATGACATGATTACGAATTAA

**FIG. 16D**

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AAGCTTTACTCGTAAAGCGAGTTGAAGGATCATATTTAGTTGCGTTTATGAGATAAGATT  
GAAAGCACGTGTAAA  
><start ORF504 (PTP)>  
ATGTTTCCCGCGCGTTGGCACAACCTATTTACAATGCGGCCAAGTTATAAAAGATTCTAAT  
CTGATATGTTTTAAACACCTTTGCGGCCCGAGTTGTTTGCCTACGTGACTAGCGAAGAA  
GATGTGTGGACCGCAGAACAGATAGTAAAACAAAACCTAGTATTGGAGCAATAATCGAT  
TTAACCAACACGTCTAAATATTATGATGGTGTGCATTTTTTGCGGGCGGGCCTGTTATAC  
AAAAAATTCAAGTACCTGGCCAGACTTTGCCGCCTGAAAGCATAGTTCAAGAATTTATT  
GACACGGTAAAAGAATTTACAGAAAAGTGTCCCGGCATGTTGGTGGGCGTGCACCTGCACA  
CACGGTATTAATCGCACCGGTTACATGGTGTGCAGATATTTAATGCACACCCTGGGTATT  
GCGCCGCAGGAAGCCATAGATAGATTGAAAAAGCCAGAGGTCACAAAATTGAAAGACAA  
AATTACGTTCAAGATTTATTAATTTAATTAATATTATTGTCATTCTTTAACAAATACTTT  
ATCCTATTTTTCAAATGTTGCGCTTCTTCCAGCGAACCAAACTATGCTTCGCTTGCTCC  
GTTTAGCTTGTTAGCCGATCAGTGGCGTTGTTCCAATCGACGGTAGGATTAGGCCGGATAT  
TCTCCACCACAATGTTGGCAACGTTGATGTTACGTTTATGCTTTTGGTTTTCCACGTACG  
TCTTTTGGCCGGTAATAGCCGTAAACGTAGTGCCGTGCGCGTCACGCACAACACCCGGAT  
GTTTGCCTTGTTCCGCGGGGTATTGAACCGCGCGATCCGACAAATCCACCCTTTGGCAA  
CTAAATCGGTGACCTGCGCGTCTTTTTTCTGCATTATTTTCGTCTTTCTTTTGCATGGTTT  
CCTGGAAGCCGGTGTACATGCGGTTTAGATCAGTCATGACGCGGTGACCTGCAAATCTT  
TGGCCTCGATCTGCTTGCTCTTGATGGCAACGATGCGTTCAATAAACTCTTGTTTTTAA  
CAAGTTCCTCGGTTTTTTGCGCCACCACCGCTTGACGCGGTTTGTGTGCTCGGTGAATG  
TCGCAATCAGCTTAGTCACCAACTGTTTGCTCTCCTCCTCCCGTTGTTTGATCGCGGGAT  
CGTACTTGCCGGTGCAGAGCACTTGAGGAATTACTTCTTCTAAAAGCCATTCTTGTAATT  
CTATGGCGTAAGGCAATTTGGACTTCATAATCAGCTGAATCACGCCGGATTAGTAATGA  
GCCCCCATTTTGGATGGTCTGCTCAAATAACGATTGTATTATTATGTCTACATGAACAC  
GTATAGCTTTATCACAACTGTATATTTTAACTGTTAGCGACGTCCTTGCCACGAACC  
GGACCTGTTGGTCGCGCTCTAGCACGTACCGCAGGTTGAACGTATCTTCTCAAATTTAA  
ATTCTCCAATTTTAACGCGAGCCA  
><start ORF984 (ORF2)>  
TTTTGATACACGTGTGTCGATTTTGCAACAACCTATTGTTTTTTAACGCAAACTAACTTA  
TTGTGGTAAGCAATAATTAAATATGGGGGAACATGCGCCGCTACAACACTCGTCGTTATG  
AACGCAGACGGCGCCGGTCTCGGCGCAAGCGGCTAAACGTGTTGCGCGTTCAACGCGGC  
AAACATCGCAAAAGCCAATAGTACAGTTTTGATTTGCA  
><start conotoxin>  
TATTAACGGCGATTTTTTAAATTATCTTATTTAATAAATAGTTATGACGCCTACAACCTCC  
CCGCCCCGCGTTGACTCGCTGCACCTCGAGCAGTTGTTGACGCCTTCTCCGTGTGGCCG  
AACACGTCGAGCGGGTGGTTCGATGACCAGCGGCGTGCCGCACGCGACGCACAAGTATCTG  
TACACCGAATGATCGTCCGGCGAAGGCACGTCGGCCTCCAAGTGGCAATATTGGCAAATT  
CGAAAATATATACAGTTGGGTGTTTGCATATCTATCGTGGCGTTGGGCATGTACGTC  
CGAACGTTGATTTGCATGCAAGCCGAAATTAAATCATTTGCGATTAGTGCATTTAAACGT  
TGTACATCCTCGCTTTTAAATCATGCCGTCGATTAAATCGCGCAATCGAGTCAAGTGATCA  
AAGTGTGGAATAATGTTTTCTTTGTATTCCCGAGTCAAGCGCAGCGGTATTTTAAACAA  
CTAGCCATCTTGTAAGTTAGTTTCA  
><start ORF453>  
  
TTTAATGCAACTTTTATCCAATAATATATT  
><start ORF327>  
ATGTATCGCACGTCAAGAATTAACAATGCGCCCGTTGTCGCATCTCAACACGACTATGAT  
AGAGATCAAATAAAGCGCGAATTAAATAGCTTGCGACGCAACGTGCACGATCTGTGCACG  
CGTTCCGGCACGAGCTTTGATTGTAATAAGTTTTTACGAAGCGATGACATGACCCCCGTA  
GTGACAACGATCACGCCCCAAAAGAACTGCCGACTACAAAATTACCGAGTATGTCGGTGAC  
GTTAAACTATTAAGCCATCCAATCGACCGTTAGTCGAATCAGGACCGCTGGTGCAGAA  
GCCGCGAAGT  
><start ORF630>



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ATGGCGAATGCATCGTATAACGTGTGGAGTCCGCTCATTAGAGCGTCATGTTTAGACAAG  
AAAGCTACATATTTAATTGATCCCGATGATTTTATTGATAAATTGACCCTAACTCCATAC  
ACGGTATTCTACAATGGCGGGGTTTTGGTCAAAATTTCCGGACTGCGATTGTACATGCTG  
TTAACGGCTCCGCCCACTATTAATGAAATTAAAAATCCAATTTTAAAAAACGCAGCAAG  
AGAAACATTTGTATGAAAGAATGCGTAGAAGGAAAGAAAAATGTCGTCGACATGCTGAAC  
AACAAGATTAATATGCCTCCGTGTATAAAAAAATATTGAACGATTTGAAAGAAAACAAT  
GTACCGCGCGGGCGGTATGTACAGGAAGAGGTTTATACTAAACTGTTACATTGCAAACGTG  
GTTTCGTGTGCCAAGTGTGAAAACCGATGTTTAATCAAGGCTCTGACGCATTTCTACAAC  
CACGACTCCAAGTGTGTGGGTGAAGTCATGCATCTTTTAATCAAATCCCAAGATGTGTAT  
AAACCACCAAACCTGCCAAAAAATGAAAACCTGTCGACAAGCTCTGTCCGTTTGCTGGCAAC  
TGCAAGGGTCTCAATCCTATTTGTAATTATTGAATAATAAAACAATTATAATGCTAAAT  
TTGTTTTTTTATTAACGATACAAACCAACGCAACAAGAACATTTGTAGTATTATCTATAA  
TTGAAAACGCGTAGTTATAATCGCTGAGGTAATATTTAAAATCATTTTCAAATGATTAC  
AGTTAATTTGCGACAATATAATTTTATTTTCACATAAACTAGACGCCTTGTCGTCTTCTT  
CTTCGTATTCTTCTCTTTTTCATTTTCTCCTCATAAAAATTAACATAGTTATTATCGT  
ATCCATATATGTATCTATCGTATAGAGTAAATTTTGTGTCATAAATATATATGTCTT  
TTTTAATGGGGTGTATAGTACCGCTGCGCATAGTTTTCGTAAATTTACAACAGTGCTAT  
TTTCTGGTAGTTCTTCGGAGTGTGTTGCTTTAATTATTAAATTTATATAATCAATGAATT  
TGGGATCGTCGGTTTTGTACAATATGTTGCCGGCATAGTACGCAGCTTCTTCTAGTTCAA  
TTACACCATTTTTTAGCAGCACCGGATTAACATAACTTTCCAAAATGTTGTACGAACCGT  
TAAACAAAAACAGTTCACCTCCCTTTTCTATACTATTGTCTGCGAGCAGTTGTTTGTGT  
TAAAAATAACAGCCA  
><start ORF603>  
TTGTAATGAGACGCACAACTAATATCACAACTGGAAATGTCTATCAATATATAGTTGC  
TGATATCATGGAGATAATTAATAATGATAACCATCTCGCAAATAAA  
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TAAGTATTTTACTGTTTTCGTAACAGTTTTTGTAAATAAAAAAACCTATAAAT  
><mutated polh start codon>  
ATTCGGATTATTCATACCGTCCCACCATCGGGCGC  
><start polylinker >  
GGATCCGCGCGCCGAATTCTAAACCACCATGGCTAGCAGGCCT  
><start of IgG>  
GACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTC  
TTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTCACA  
TGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGAC  
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC  
CGTGTGGTCAGCGTCTCACCCTGTCACCAAGGACTGGCTGAATGGCAAGGAGTACAAG  
TGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA  
GGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAAGAGATGACCAAG  
AACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAG  
TGGGAGAGCAATGGGCAGCCGGAGAACAATAAGACCACGCCTCCCGTGCTGGACTCC  
GACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGG  
AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC  
CTCTCCCTGTCTCCGGGTAAA  
><end of IgG>  
TGACATAGGG  
><untranslated His tag>  
CATCATCATCATCATCATCATTAATTCTAGACTAGTCTGCAGATC  
><end polylinker>  
T

**FIG. 17B**

SUBSTITUTE SHEET ( rule 26 )

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&gt;&lt;polh coding sequences&gt;

GATCCTTTTCTGGGACCCGGCAAGAACCAAAAACCTCACTCTCTTCAAGGAAATCCGTAAT  
GTTAAACCCGACACGATGAAGCTTGTCTGTTGGATGGAAAGGAAAAGAGTTCTACAGGGAA  
ACTTGGACCCGCTTCATGGAAGACAGCTTCCCCATTGTTAACGACCAAGAAGTGATGGAT  
GTTTTCTTGTGTCAACATGCGTCCCCTAGACCCAACCGTTGTTACAAATTCCTGGCC  
CAACACGCTCTGCGTTGCGACCCGACTATGTACCTCATGACGTGATTAGGATCGTCGAG  
CCTTCATGGGTGGGCAGCAACAACGAGTACCGCATCAGCCTGGCTAAGAAGGGCGGCGGC  
TGCCCAATAATGAACCTTCACTCTGAGTACACCAACTCGTTCGAACAGTTCATCGATCGT  
GTCATCTGGGAGAACTTCTACAAGCCCATCGTTTACATCGGTACCGACTCTGCTGAAGAG  
GAGGAAATTCCTCTGAAGTTTCCCTGGTGTTCAAAGTAAAGGAGTTTGCACCAGACGCA  
CCTCTGTTCACTGGTCCGGCGTATTAAAACACGATACATTGTTATTAGTACATTTATTAA  
GCGCTAGATTCTGTGCGTTGTTGATTACAGACAATTGTTGTACGTATTTTAATAATTCA  
TTAAATTTATAATCTTTAGGGTGGTATGTTAGAGCGAAAATCAAATGATTTTCAGCGTCT  
TTATATCTGAATTTAAATATTAATCCTCAATAGATTTGTAAAATAGGTTTCGATTAGTT  
TCAAACAAGGGTTGTTTTTCCGAACCGATGGCTGGACTATCTAATGGATTTTCGCTCAAC  
GCCACAAAACCTTGCCAAATCTTGTAGCAGCAATCTAGCTTGTGCGATATTCGTTGTGTT  
TTGTTTTGTAATAAAGGTTTCGACGTCGTTCAAATATTATGCGCTTTTGTATTTCTTTCA  
TCACTGTCGTTAGTGACAATTGACTCGACGTAAACACGTTAAATAAAGCTTGGACATAT  
TTAACATCGGGCGTGTAGCTTTATTAGGCCGATTATCGTCGTCGTCCTCAACCCCTCGTCG  
TTAGAAGTTGCTTCCGAAGACGATTTTGCCATAGCCACACGACGCTATTAATTGTGTGCG  
GCTAACACGTCGCGGATCAAATTTGTAGTTGAGCTTTTGGAAATTATTTCTGATTGCGGG  
CGTTTTTGGGCGGGTTTCAATCTAACTGTGCCCGATTTTAATTACAGACAACACGTTAGAA  
AGCGATGGTGCAGGCGGTGGTAACATTTAGACGGCAAATCTACTAATGGGCGGCGGTGGT  
GGAGCTGATGATAAATCTACCATCGGTGGAGGCGCAGGCGGGGCTGGCGGCGGAGGCGGA  
GGCGGAGGTGGTGGCGGTGATGCAGACGGCGGTTTAGGCTCAAATGTCTCTTTAGGCAAC  
ACAGTCGGCACCTCAACTATTGTACTGGTTTCGGGCGCCGTTTTTGGTTTTGACCGGTCTG  
AGACGAGTGCGATTTTTTTTCGTTTCTAATAGCTTCCAACAATTGTTGTCTGTCGCTCTAA  
GGTGCAGCGGGTTGAGGTTCCGTCGGCATTTGGTGGAGCGGGCGGCAATTCAGACATCGAT  
GGTGGTGGTGGTGGTGGAGGCGCTGGAATGTTAGGCACGGGAGAAAGGTGGTGGCGGCGGT  
GCCGCCGCTATAATTTGTTCTGGTTTAGTTTGTTCGCGCACGATTGTGGGCACCGGCGCA  
GAATCAATATTATAATTGGAATACAAATCGTAAAAATCTGCTATAAGCATTGTAATTTTCG  
CTATCGTTTACCGTGCCGATATTTAACAACCGCTCAATGTAAGCAATTGTATTGTAAAGA  
GATTGTCTCAAGCTCCGCACGCGGATAACAAGCCTTTTCATTTTACTACAGCATTGTAG  
TGGCGAGACACTTCGCTGTCTGTCGACGTACATGTATGCTTTGTTGTCAAAAACGTCGTTG  
GCAAGCTTTAAAATATTTAAAAGAACATCTCTGTTTCAGCACCCTGTGTTGTGCGTAAATG  
TTGTTTTTGATAATTTGCGCTTCCGCAGTATCGACACGTTCAAAAATTTGATGCGCATCA  
ATTTTGTGTTCTTATTATTGAATAAATAAGATTGTACAGATTCATATCTACGATTTCGTC  
><start ORF588>

A

&gt;&lt;start ORF1629&gt;

TGGCCACCACAAATGCTACGCTGCAAACGCTGGTACAATTTTACGAAAACCTGCAAAAACG  
TCAAAAACCTCGGTATAAAATAATCAACGGGCGCTTTGGCAAAATATCTATTTTATCGCACA  
AGCCCCTAGCAAATTGTATTTGCAGAAAACAATTTCCGGCGCACAATTTTAAACGCTGACG  
AAATAAAAGTTTACCAGTTAATGAGCGACCACCCAAATTTTATAAAAATCTATTTTAATC  
ACGGTTCCATCAACAACCAAGTGATCGTGATGGACTACATTGACTGTCCCGATTTATTG  
AAACACTACAAATTAAAGGCGAGCTTTCGTACCAACTTGTAGCAATATTATTAGACAGC  
TGTGTGAAGCGCTCAACGATTTGCACAAGCAATTTTCATACACAACGAACTCG  
AAAATGTCTTATATTTTGAAGCACTTGATCGCGTGTATGTTTGGGATTACGGATTGTGCA  
AACACGAAAACCTCACTTAGCGTGCACGACGGCAGCTTGGAGTATTTTAGTCCGGAAAAAA  
TTCGACACACAACATATGCACGTTTCGTTTACTGGTACGCGGCGTGTAAACATACAAGTT  
GCTAACCGGCGG

**FIG. 17C**

SUBSTITUTE SHEET ( rule 26 )

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&gt;&lt;end of polh locus fragment&gt;

TTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCACACA  
CAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACT  
CACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCTGTGCCAGCT  
GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTG

&gt;&lt;border ColE1 origin&gt;

GGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCCGGCTGCGGCGAG  
CGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAG  
GAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGC  
TGGCGTPTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTC  
AGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC  
TCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTT  
CGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCTG  
TTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTAT  
CCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAG  
CCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGT  
GGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGC  
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTA  
GCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAG  
ATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACCTACGTTAAGGGA  
TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT

&gt;&lt;border ColE1 origin&gt;

AAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAG  
TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCAT  
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCC  
CAGTGCCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAA  
CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCCTGCAACTTTATCCGCCTCCATCCA  
GTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCAA  
CGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATT  
CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGC  
GGTTAGCTCCTTCGGTCCCTCCGATCGTTGTGAGAAGTAAGTTGGCCGCAGTGTTATCACT  
CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTAAGATGCTTTTC  
TGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTG  
CTCTTGCCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCT  
CATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATC  
CAGTTTCGATGTAACCCACTCGTGCAACCAACTGATCTTCAGCATCTTTTACTTTTACCAG  
CGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAAGGGAATAAGGGCGAC  
ACGGAAATGTTGAATACTCA

&gt;&lt;Start Amp&gt;

TACTCTTCCTTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGAT  
ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAA  
AAGTGCCACCTGACGTCTAAGAAACCATTTATTATCATGACATTAACCTATAAAAAATAGGC  
GTATCACGAGGCCCTTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACA  
TGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCC  
GTACAGGCGCGTCAGCGGGTGTGCGGGGTGTCGGGGCTGGCTTAACCTATGCGGCATCAG  
AGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAGATGCGTAAG  
GAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCG  
ATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCG  
ATTAAGTTGGGTAACGCCAGGGTTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGC  
C

**FIG. 17D**

SUBSTITUTE SHEET ( rule 26 )

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AAGCTTTACTCGTAAAGCGAGTTGAAGGATCATATTTAGTTGCGTTTATGAGATAAGATT  
 GAAAGCACGTGTAAA  
 ><start ORF504 (PTP)>  
 ATGTTTCCGCGCGTTGGCACAAC TATTTACAATGCGGCCAAGTTATAAAAGATTCTAAT  
 CTGATATGTTTTAAACACCTTTGCGGCCGAGTTGTTTTCGTACGTGACTAGCGAAGAA  
 GATGTGTGGACCGCAGAACAGATAGTAAACAAAACCCTAGTATTGGAGCAATAATCGAT  
 TTAACCAACACGTCTAAATATTATGATGGTGTGCATTTTTTTCGCGGCGGGCCTGTTATAC  
 AAAAAAATTCAAGTACCTGGCCAGACTTTGCCGCCTGAAAGCATAGTTCAAGAATTTATT  
 GACACGGTAAAAGAATTTACAGAAAAGTGTCCCGGCATGTTGGTGGGCGTGCACTGCACA  
 CACGGTATTAATCGCACCGGTTACATGGTGTGCAGATATTTAATGCACACCCTGGGTATT  
 GCGCCGCAGGAAGCCATAGATAGATTTCGAAAAGCCAGAGGTCACAAAATTGAAAGACAA  
 AATTACGTTCAAGATTTATTAATTTAATTAATATTATTTGCATTCTTTAACAATACTTT  
 ATCCTATTTTCAAATTGTTGCGCTTCTTCCAGCGAACCAAACTATGCTTCGCTTGCTCC  
 GTTTAGCTTGTAGCCGATCAGTGGCGTTGTTCCAATCGACGGTAGGATTAGGCCGGATAT  
 TCTCCACCACAATGTTGGCAACGTTGATGTTACGTTTATGCTTTTGGTTTTCCACGTACG  
 TCTTTTGGCCGGTAATAGCCGTAAACGTAGTGCCGTCGCGCGTCACGCACAACACCGGAT  
 GTTTGCGCTTGTCGCGGGGTATTGAACCGCGCGATCCGACAAATCCACCACCTTTGGCAA  
 CTAAATCGGTGACCTGCGCGTCTTTTTTCTGCATTATTTCTGCTTTCTTTTGCATGGTTT  
 CCTGGAAGCCGGTGTACATGCGGTTTAGATCAGTCATGACGCGCGTGACCTGCAAATCTT  
 TGGCCTCGATCTGCTTGTCCTTGATGGCAACGATGCGTTCAATAAACTCTTGTTTTTTAA  
 CAAGTTCCTCGGTTTTTTGCGCCACCACCGCTTGACGCGGTTGTGTGCTCGGTGAATG  
 TCGCAATCAGCTTAGTCACCAACTGTTTGCTCTCCTCCTCCCGTTGTTTGATCGCGGGAT  
 CGTACTTGCCGGTGCAGAGCACTTGAGGAATTACTTCTTCTAAAAGCCATTCTTGTAATT

CTATGGCGTAAGGCAATTTGGACTTCATAATCAGCTGAATCACGCCGGATTTAGTAATGA  
 GCACTGTATGCGGCTGCAAATACAGCGGGTCGCCCTTTTCACGACGCTGTTAGAGGTAG  
 GGCCCCCATTTTGGATGGTCTGCTCAAATAACGATTTGTATTTATTGTCTACATGAACAC  
 GTATAGCTTTATCACAACCTGTATATTTAACTGTTAGCGACGTCCTTGGCCACGAACC  
 GGACCTGTTGGTTCGCGCTCTAGCACGTACCGCAGGTTGAACGTATCTTCTCCAAATTTAA  
 ATTCTCCAATTTTAACGCGAGCCA  
 ><start ORF984 (ORF2)>  
 TTTTGATACACGTGTGTCGATTTTGCAACAAC TATTTGTTTTTTAACGCAAACTAACTTA  
 TTGTGGTAAGCAATAATTAAATATGGGGGAACATGCGCCGCTACAACACTCGTCGTTATG  
 AACGCAGACGGCGCCGGTCTCGGCGCAAGCGGCTAAAACGTGTTGCGCGTTCAACGCGGC  
 AAACATCGCAAAAGCCAATAGTACAGTTTGTATTTGCA  
 ><start conotoxin>  
 TATTAACGGCGATTTTTTAAATTATCTTATTTAATAAATAGTTATGACGCCTACAACCTCC  
 CCGCCCGGTTGACTCGCTGCACCTCGAGCAGTTTCGTTGACGCCTTCCTCCGTGTGGCCG  
 AACACGTCGAGCGGGTGGTTCGATGACCAGCGGCGTGCCGCACGCGACGCACAAGTATCTG  
 TACACCGAATGATCGTCGGGCGAAGGCACGTCGGCCCTCCAAGTGGCAATATTGGCAAATT  
 CGAAAATATATACAGTTGGGTGTTTTCGCATATCTATCGTGGCGTTGGGCATGTACGTC  
 CGAACGTTGATTTGCATGCAAGCCGAAATTAATCATTCGATTAGTGCGATTAAAACGT  
 TGTACATCCTCGCTTTTAATCATGCCGTCGATTAAATCGCGCAATCGAGTCAAGTGATCA  
 AAGTGTGGAATAATGTTTTCTTTGTATTCCCGAGTCAAGCGCAGCGCGTATTTTAACAAA  
 CTAGCCATCTTGTAAGTTAGTTTCA

**FIG. 18A**

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><start ORF453>  
TTTAATGCAACTTTATCCAATAATATATT  
><start ORF327>  
ATGTATCGCACGTCAAGAATTAACAATGCGCCCGTTGTCGCATCTCAACACGACTATGAT  
AGAGATCAAATAAAGCGCGAATTAAATAGCTTGCGACGCAACGTGCACGATCTGTGCACG  
CGTTCCGGCAGGAGCTTTGATTGTAATAAGTTTTTACGAAGCGATGACATGACCCCCGTA  
GTGACAACGATCAGCCCCAAAAGAACTGCCGACTACAAAATTACCGAGTATGTCGGTGAC  
GTTAAAACATTAAGCCATCCAATCGACCGTTAGTCGAATCAGGACCGCTGGTGCGAGAA  
GCCGCGAAGT  
><start ORF630>  
ATGGCGAATGCATCGTATAACGTGTGGAGTCCGCTCATTAGAGCGTCATGTTTAGACAAG  
AAAGCTACATATTTAATTGATCCCGATGATTTTATTGATAAATTGACCCTAACTCCATAC  
ACGGTATTCTACAATGGCGGGGTTTTGGTCAAAAATTTCCGGACTGCGATTGTACATGCTG  
TTAACGGCTCCGCCCCACTATTAATGAAATTAATAAATTTCCAATTTTAAAAAACGCAGCAAG  
AGAAACATTTGTATGAAAGAATGCGTAGAAGGAAAGAAAAATGTCGTCGACATGCTGAAC  
AACAAGATTAATATGCCTCCGTGTATAAAAAAATATTGAACGATTTGAAAGAAAACAAT  
GTACCGCGCGGGCGGTATGTACAGGAAGAGGTTTATACTAACTGTTACATTGCAAACGTG  
GTTTCGTGTGCCAAGTGTGAAAACCGATGTTTAATCAAGGCTCTGACGCATTTCTACAAC  
CAGACTCCAAGTGTGTGGGTGAAGTCATGCATCTTTTAATCAAATCCCAAGATGTGTAT  
AAACCACCAAACCTGCCAAAAAATGAAAACCTGTCGACAAGCTCTGTCCGTTTGCTGGCAAC  
TGCAAGGGTCTCAATCCTATTTGTAATTATTGAATAATAAACAAATATAAATGCTAAAT  
TTGTTTTTTTATTAACGATACAAACCAAACGCAACAAGAACATTTGTAGTATTATCTATAA  
TTGAAAACGCGTAGTTATAATCGCTGAGGTAATATTTAAATCATTTTCAAATGATTAC  
AGTTAATTTGCGACAATATAATTTTATTTTCACATAAACTAGACGCCTTGTCGTCTTCTT  
CTTCGTATTCCTTCTCTTTTTTCATTTTTCTCCTCATAAAAATTAACATAGTTATTATCGT  
ATCCATATATGTATCTATCGTATAGAGTAAATTTTTTGTGTCATAAATATATATGTCTT  
TTTTAATGGGGTGTATAGTACCGCTGCGCATAGTTTTTCTGTAAATTTACAACAGTGCTAT  
TGGGATCGTCGGTTTTGTACAATATGTTGCCGGCATAGTACGCAGCTTCTTCTAGTTCAA  
TTACACCATTTTTTTAGCAGCACCGATTAAACATAACTTTCCAAAATGTTGTACGAACCGT  
TAAACAAAACAGTTCACCTCCCTTTTCTATACTATTGTCTGCGAGCAGTTGTTTGTGT  
TAAAAATAACAGCCA  
><start ORF603>  
TTGTAATGAGACGCACAACTAATATCACAACTGGAAATGTCTATCAATATATAGTTGC  
TGATATCATGGAGATAATTAAATGATAACCATCTCGCAAATAAA  
><start of polh transcription>  
TAAGTATTTTACTGTTTTCGTAACAGTTTTTGTAAATAAAAAACCTATAAAT  
><mutated polh start codon>  
ATTCGGATTATTTCATACCGTCCCACCATCGGGCGC  
><start polylinker >  
GGATCCGCGGCCGCGAATTCTAAACCACCATGGGCAGCTGCCCCGGG  
><His tag>  
CATCATCATCATCATCATCATTAATTCTAGACTAGTCTGCAGATC  
><end polylinker>  
T

**FIG. 18B**

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&gt;&lt;polh coding sequences&gt;

GATCCTTTCTGGGACCCGGCAAGAACCAAAAACCTCACTCTCTTCAAGGAAATCCGTAAT  
GTTAAACCCGACACGATGAAGCTTGTCTGTTGGATGGAAAGGAAAGAGTTCTACAGGGAA  
ACTTGGACCCGCTTCATGGAAGACAGCTTCCCCATTGTTAACGACCAAGAAGTGATGGAT  
GTTTTCTTGTGTCAACATGCGTCCCCTAGACCCCAACCGTTGTTACAAATTCCTGGCC  
CAACACGCTCTGCGTTGCGACCCCGACTATGTACCTCATGACGTGATTAGGATCGTCGAG  
CCTTCATGGGTGGGCGAGCAACAACGAGTACCGCATCAGCCTGGCTAAGAAGGGCGGCGGC  
TGCCCAATAATGAACCTTCACTCTGAGTACACCAACTCGTTCGAACAGTTTCATCGATCGT  
GTCATCTGGGAGAACTTCTACAAGCCCATCGTTTACATCGGTACCGACTCTGCTGAAGAG  
GAGGAAATTCCTCTGAAGTTTCCCTGGTGTCAAAGTAAAGGAGTTTGCACCAGACGCA  
CCTCTGTTCACTGGTCCGGCGTATTAACACAGATACATTGTTATTAGTACATTTATTAA  
GCGCTAGATTCTGTGCGTTGTTGATTACAGACAATTGTTGTACGTATTTTAATAATTCA  
TTAAATTTATAATCTTTAGGGTGGTATGTTAGAGCGAAATCAAATGATTTTCAGCTCT  
TTATATCTGAATTTAAATATTAAATCCTCAATAGATTGTAAATAGGTTTCGATTAGTT  
TCAAACAAGGGTTGTTTTTCCGAACCGATGGCTGACTATCTAATGGATTTTCGCTCAAC  
GCCACAAAACCTGCCAAATCTGTAGCAGCAATCTAGCTTTGTCGATATTCGTTTGTGTT  
TTGTTTTGTATAAAGGTTTCGACGTCGTTCAAATATTATGCGCTTTTGTATTTCTTTCA  
TTACATGTCGTTAGTGTACAATTGACTCGACGTAAACACGTTAAATAAAGCTTGGACATAT  
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GCCGCCGGTATAATTTGTTCTGGTTTAGTTTGTTCGCGCACGATTGTGGGCACCGGCGCA  
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AATTCAATATTATAATTGGAATACAAATCGTAAAAATCTGCTATAAGCATTGTAATTTTCG  
CTATCGTTTACCGTGCCGATATTTAACAACCGCTCAATGTAAGCAATTGTATTGTAAAGA  
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A

&gt;&lt;start ORF1629&gt;

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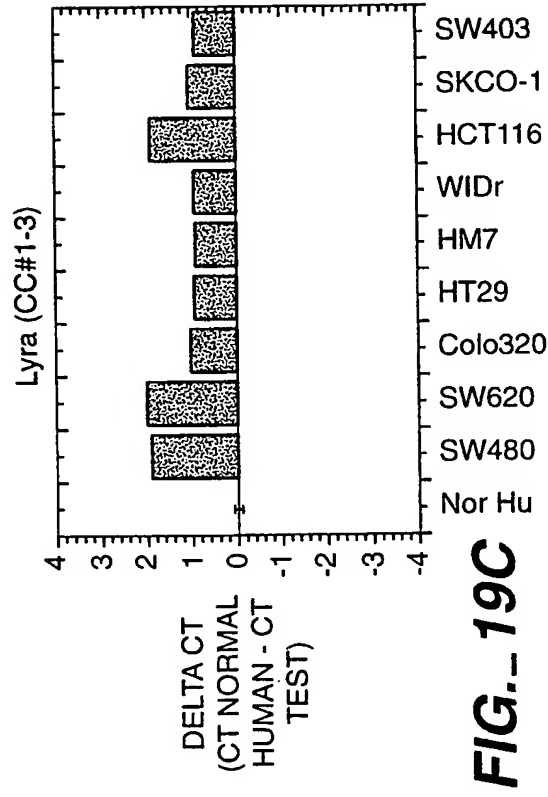
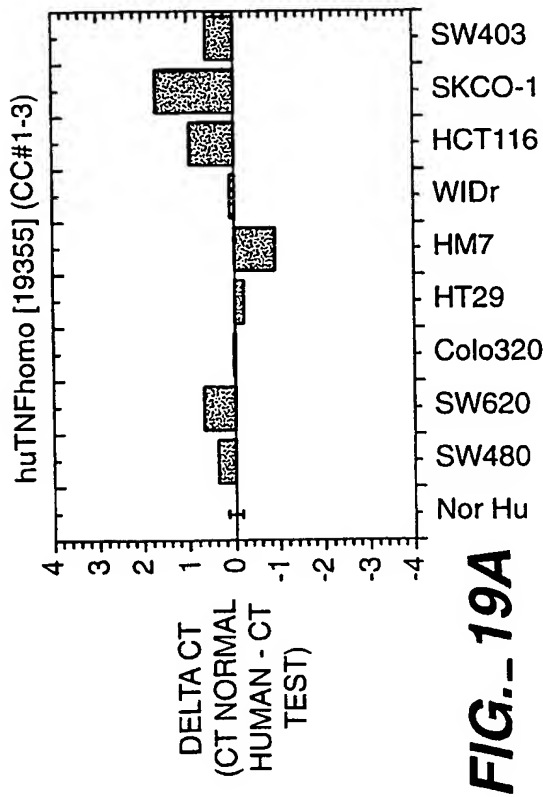
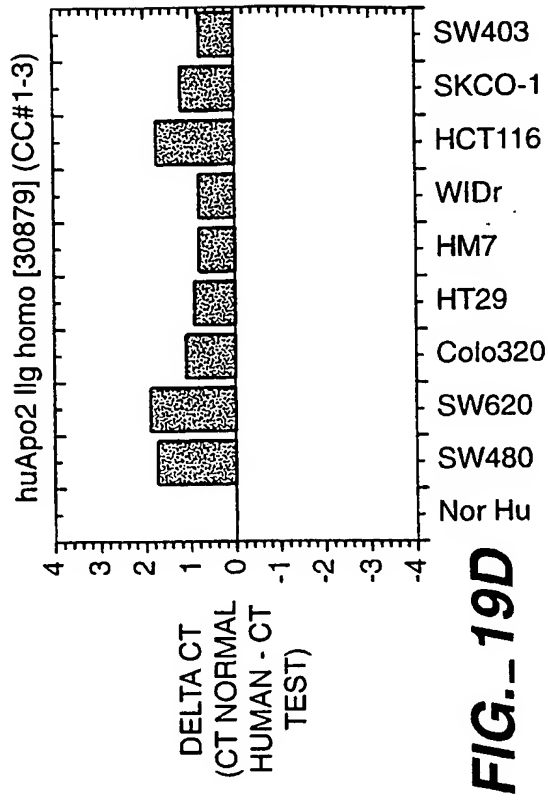
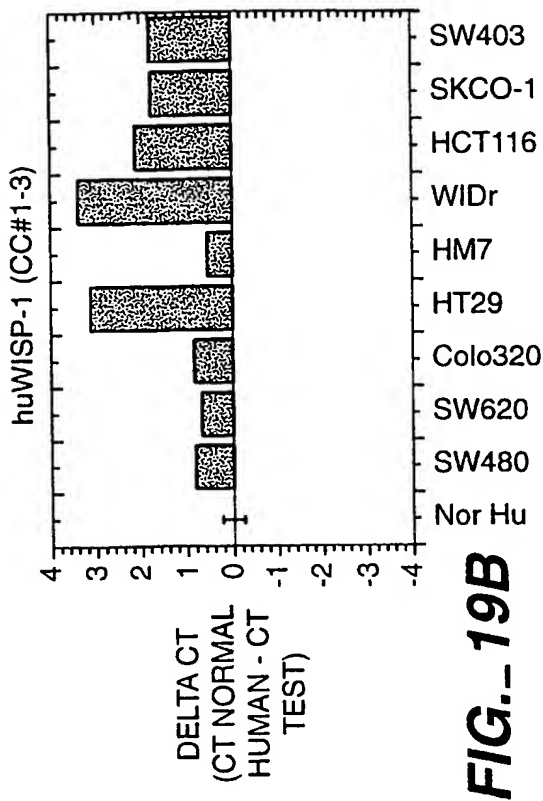
**FIG. 18C**

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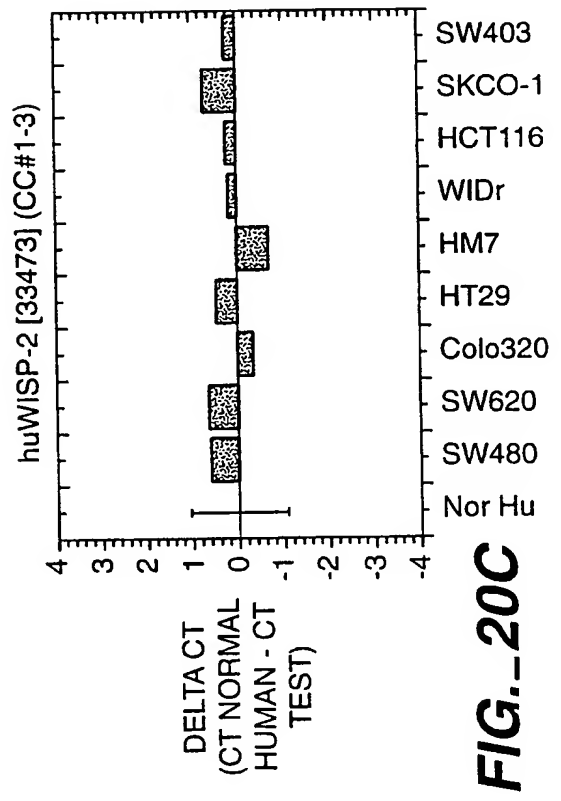
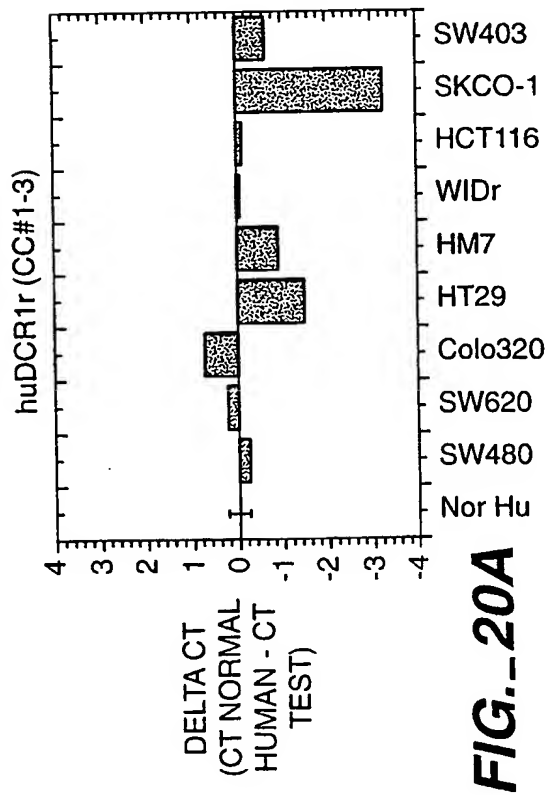
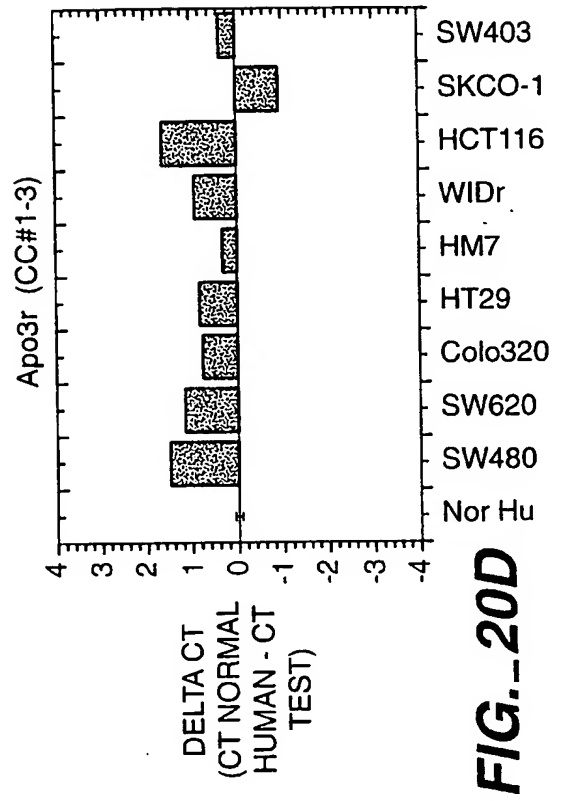
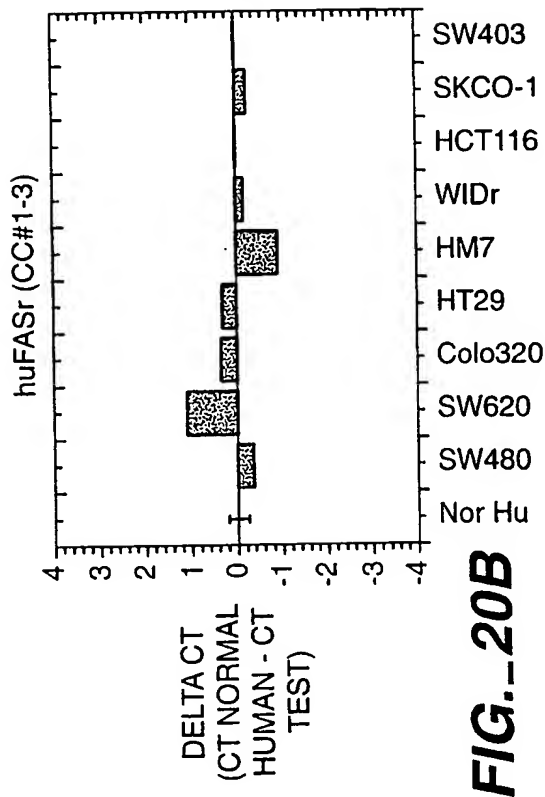
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GAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGC  
TGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTC  
AGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCC  
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GCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAG  
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AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCC  
CAGTGCTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAA  
CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTTCAACTTTATCCGCCTCCATCCA  
GTCTATTAATTTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCAA  
CGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATT  
CAGTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGC  
GGTAGCTCCTTCGGTCCCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTTATCACT  
CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTAAGATGCTTTTC  
TGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTG  
CTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCT  
CATCATTGGAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATC  
CAGTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTTACTTTCACCAG  
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GTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACA  
TGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCC  
GTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGCGGGCTGGCTTAACTATGCGGCATCAG  
AGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACCGCACAGATGCGTAAG  
GAGAAAATACCGCATCAGGCGCCATTGCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCG  
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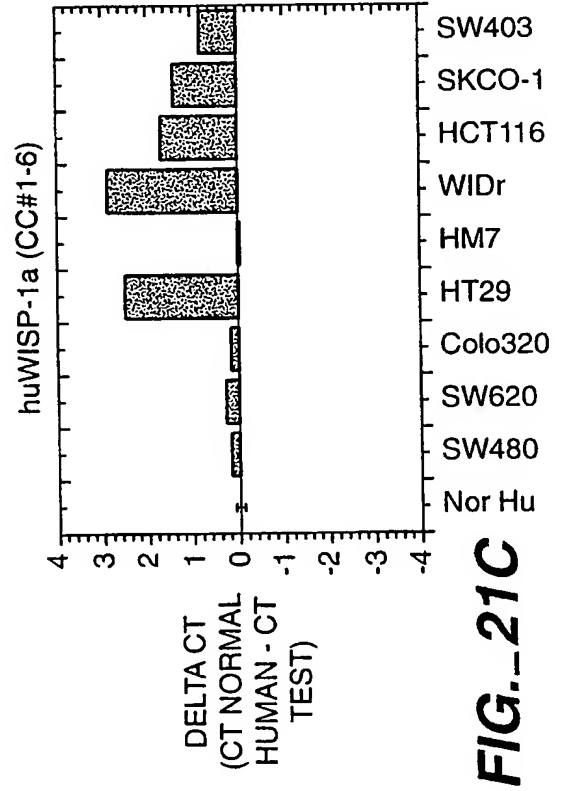
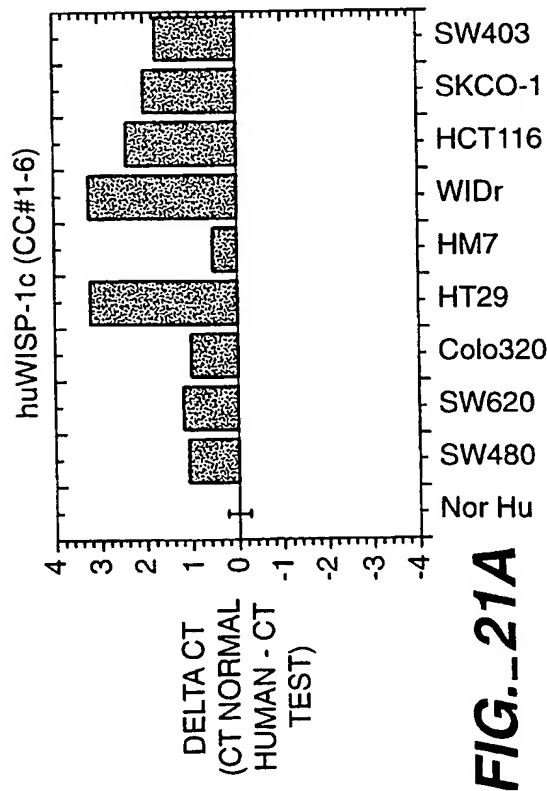
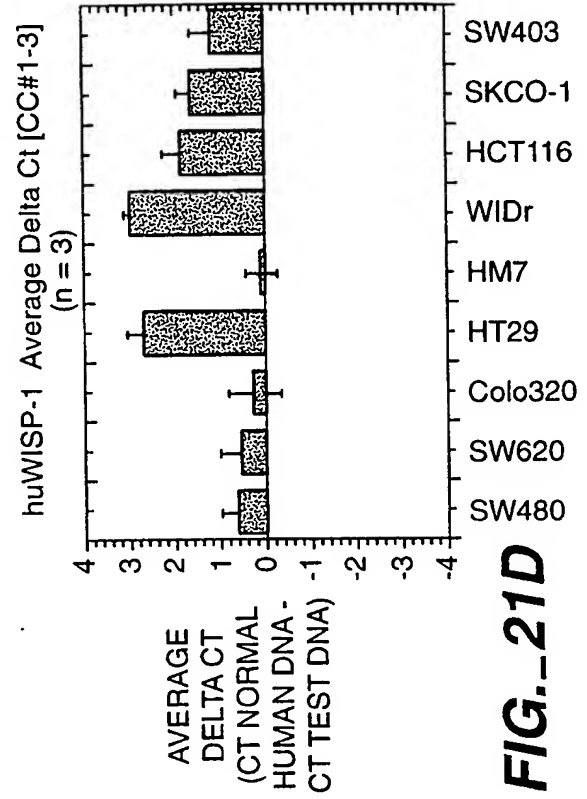
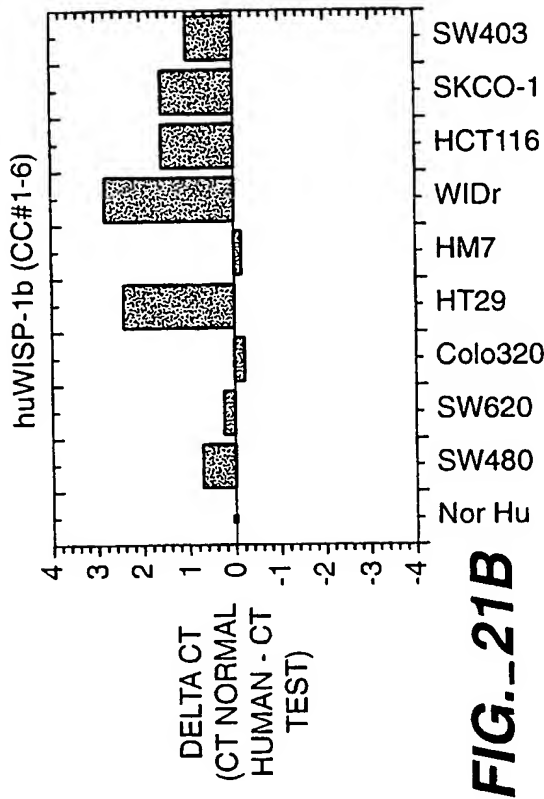
**FIG. 18D****SUBSTITUTE SHEET (RULE 26)**

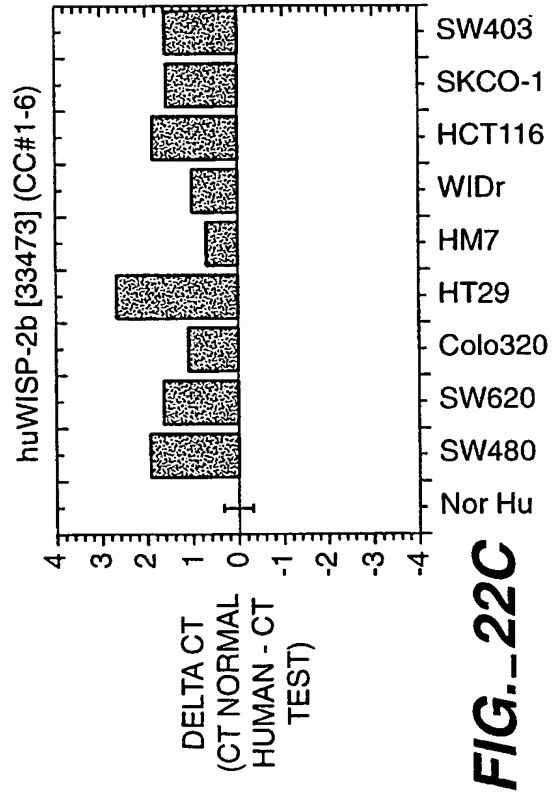
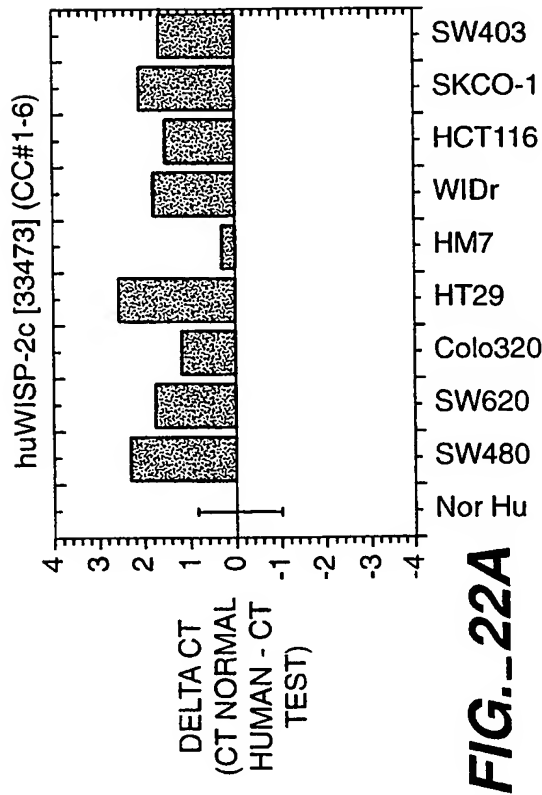
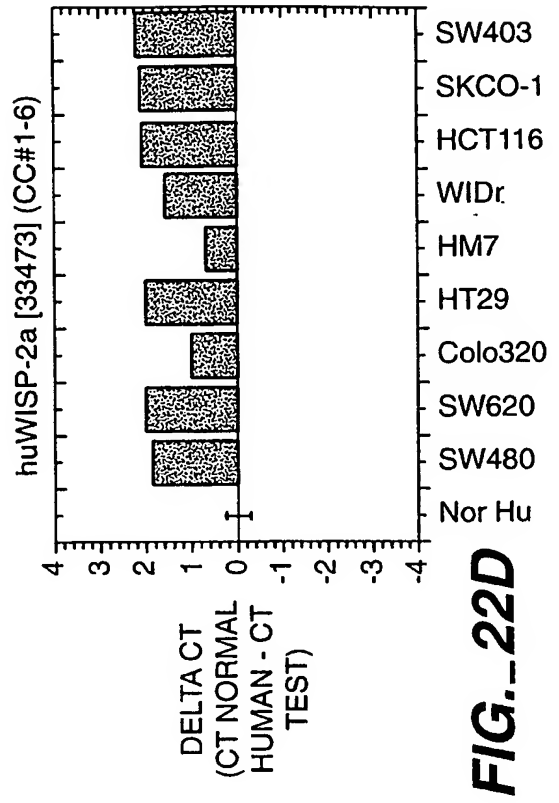
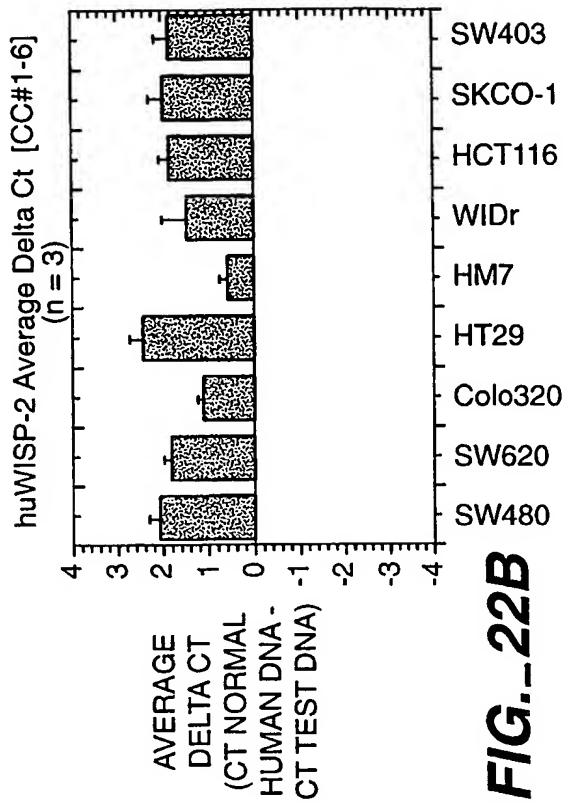


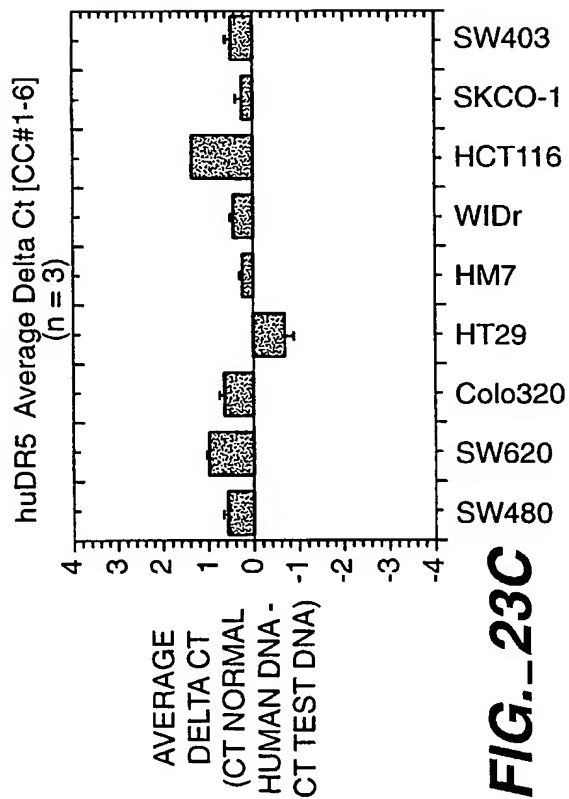
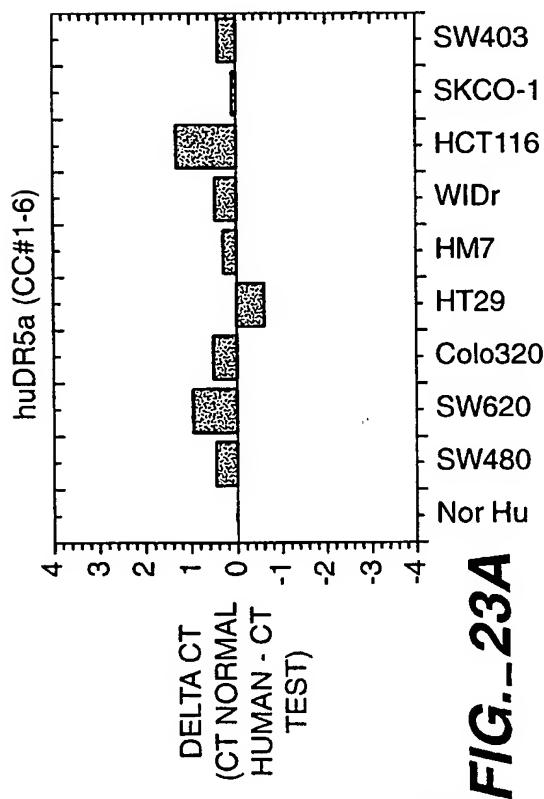
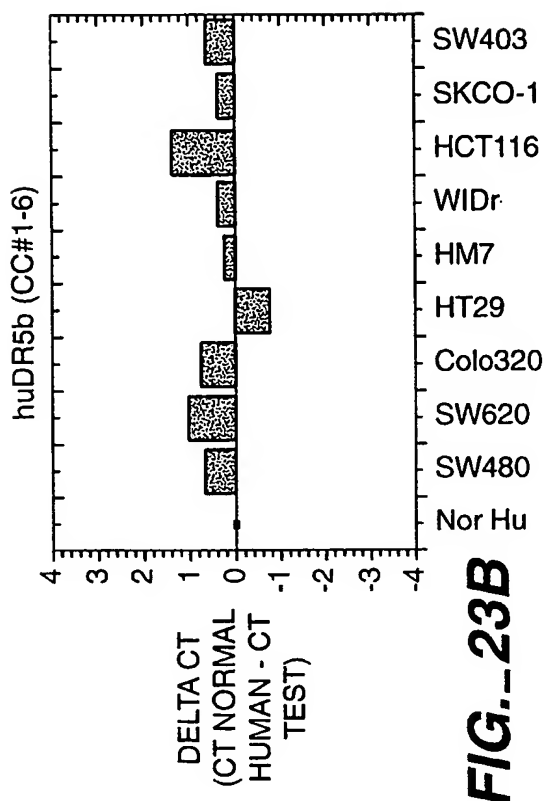


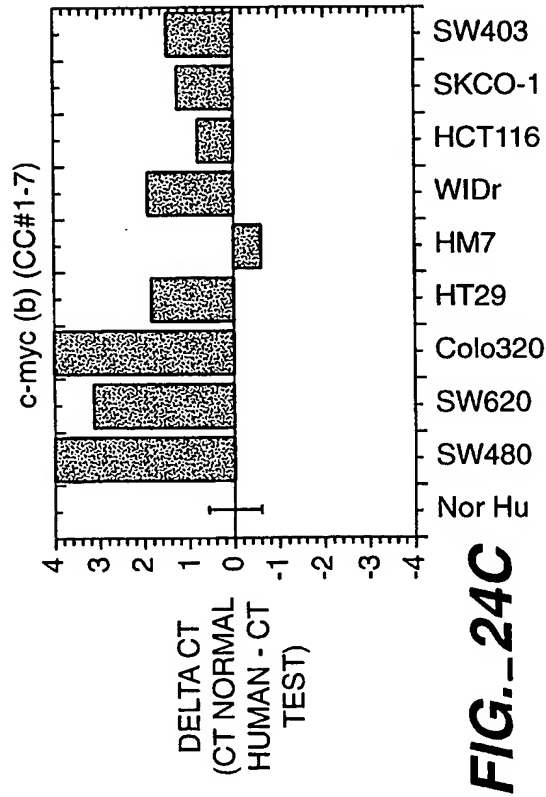
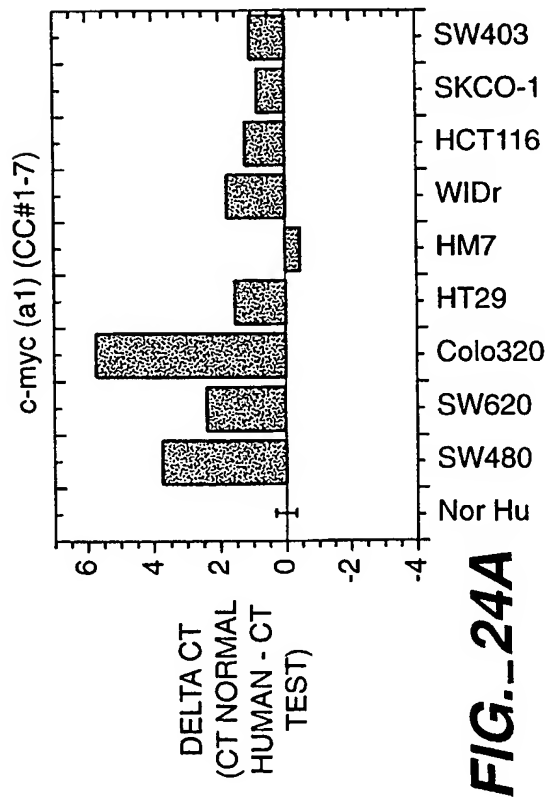
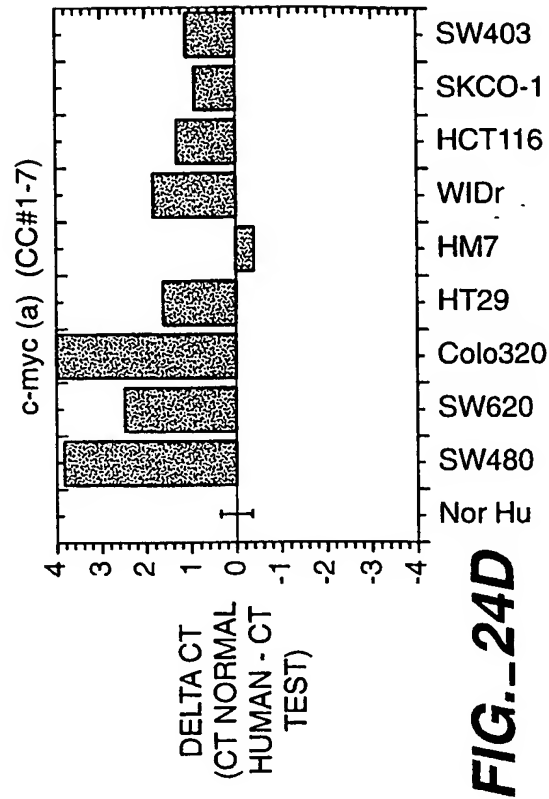
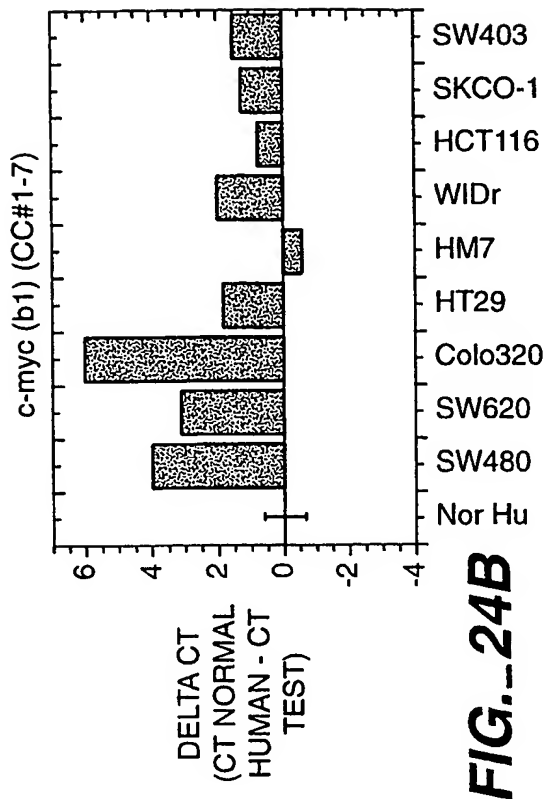


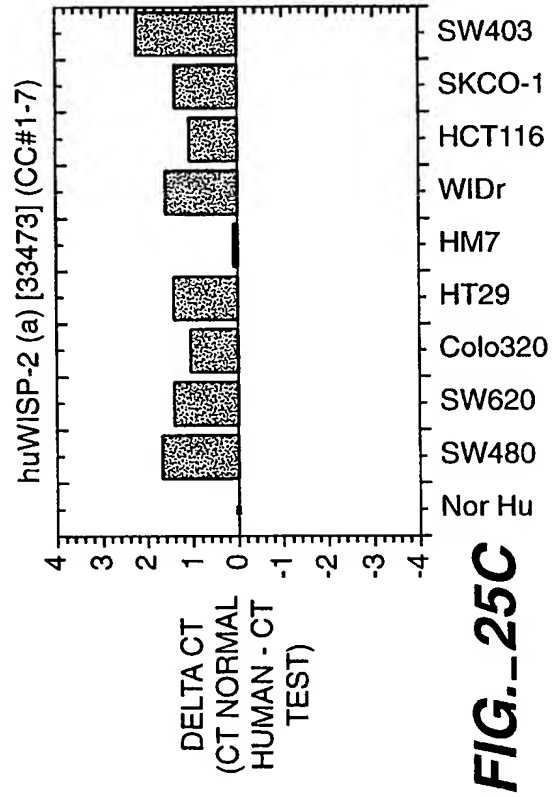
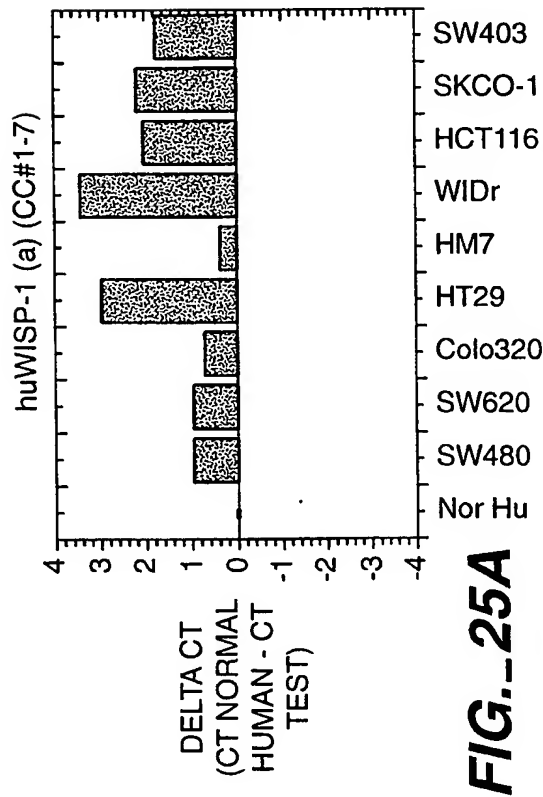
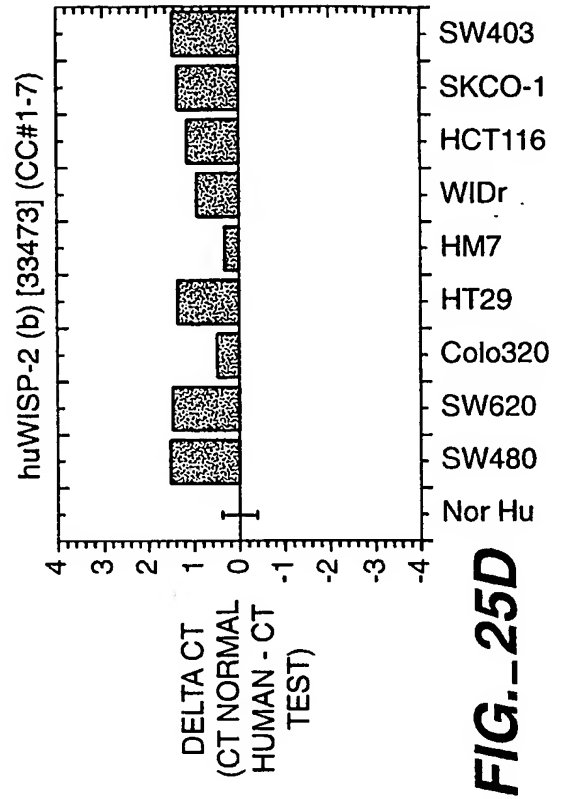
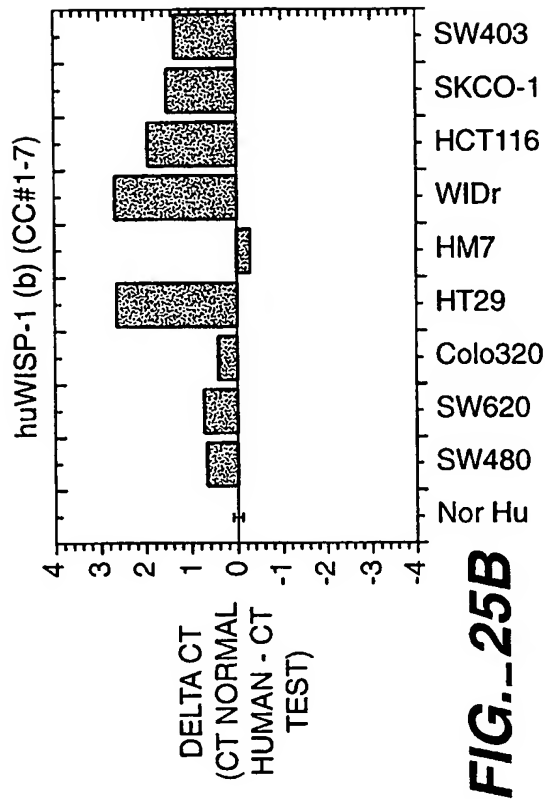












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5' -GCCAGTCTGGGCCCAGCTCCCCGAGAGGTGGTCGGATCCTCTGGGCTGCTCGGTCGATG  
CCTGTGCCACTGACGTCCAGGCATGAGGTGGTTCCTGCCCTGGACGCTGGCAGCAGTGAC  
AGCAGCAGCCGCCAGCACCGTCCTGGCCACGGCCCTCTCTCCAGCCCCCTACGACCATGGA  
CTTTACCCCAGCTCCACTGGAGGACACCTCCTCACGCCCCCAATTCTGCAAGTGGCCATG  
TGAGTGCCCGCCATCCCCACCCGCTGCCCCGCTGGGGGTGAGCCTCATCACAGATGGCTG  
TGAGTGCTGTAAGATGTGCGCTCAGCAGCTTGGGGACAAC TGACGGAGGCTGCCATCTG  
TGACCCCCACCGGGGCTCTACTGTGACTACAGCGGGGACCGCCGAGAGGTGGTTCGGTG  
TGGGCTGCGTCCTGGATGGGGTGCGCTACAACAACGGCCAGTCTTCCAGCCTAACTGCA  
AGTACAAC TGACGTGCATCGACGGCGCGGTGGGCTGCACACCACTGTGCCTCCGAGTGC  
GCCCCCGCGTCTCTGGTGCCCCCACCCGCGGCGCGTGAGCATACCTGGCCACTGCTGTG  
AGCAGTGGGTATGTGAGGACGACGCCAAGAGGCCACGCAAGACCGCACCCCGTGACACAG  
GAGCCTTCGATGCTGTGGGTGAGGTGGAGGCATGGCACAGGAACTGCATAGCCTACACAA  
GCCCCCTGGAGCCCTTGCTCCACCAGCTGCGGCCCTGGGGGTCTCCACTCGGATCTCCAATG  
TTAACGCCCAGTGCTGGCCTGAGCAAGAGAGCCGCCTCTGCAACTTGCGGCCATGCGATG  
TGGACATCCATACACTCATTAAGGCAGGGAAGAAGTGTCTGGCTGTGTACCAGCCAGAGG  
CATCCATGAACCTCACACTTGCGGGCTGCATCAGCACACGCTCCTATCAACCCAAGTACT  
GTGGAGTTTGCATGGACAATAGGTGCTGCATCCCCTACAAGTCTAAGACTATCGACGTGT  
CCTTCCAGTGTCTGATGGGCTTGGCTTCTCCCGCCAGGTCTATGGATTAATGCCTGCT  
TCTGTAACCTGAGCTGTAGGAATCCCAATGACATCTTTGCTGACTTGGAATCCTACCCTG  
ACTTCTCAGAAATTGCCAACTAGGCAGGCACAAATCTTGGGTCTTGGGGACTAACCCAAT  
GCCTGTGAAGCAGTCAGCCCTTATGGCCAATAACTTTTACCAATGAGCCTTAGTTACCC  
TGATCTGGACCCTTGGCCTCCATTTCTGTCTCTAACCATTCAAATGACGCCTGATGGTGC  
TGCTCAGGCCCATGCTATGAGTTTTCTCCTTGATATCATTCAGCATCTACTCTAAAGAAA  
AATGCCTGTCTCTAGCTGTTCTG

**FIG.\_26**

5' -TTTAATTAAACCCCCAAGGGCTGCGGAAGGAGCATATCTGGTGCTCCTGATGGGCCGGCC  
AGTCTGGGCCCAGCTCCCCGAGAGGTGGTCGGATCCTCTGGGCTGCTCGGTCGATGCCT  
GTGCCACTGACGTCCAGGCATGAGGTGGTTCCTGCCCTGGACGCTGGCAGCAGTGACAGC  
AGCAGCCGCCAGCACCGTCCTGGCCACGGCCCTCTCTCCAGCCCCCTACGACCATGGACTT  
TACCCCAGCTCCACTGGAGGACACCTCCTCACGCCCCCAATTCTGCAAGTGGCCATGTGA  
GTGCCCCGCCATCCCCACCCGCTGCCCCGCTGGGGGTGAGCCTCATCACAGATGGCTGTGA  
GTGCTGTAAGATGTGCGCTCAGCAGCTTGGGGACAAC TGACGGAGGCTGCCATCTGTGA  
CCCCCACCGGGGCTCTACTGTGACTACAGCGGGGACCGCCGAGGTACGCAATAGGAGT  
GTGTGCACGCAGGGAAGAAGTGTCTGGCTGTGTACCAGCCAGAGGCATCCATGAACCTTCA  
CACTTGCGGGCTGCATCAGCACACGCTCCTATCAACCCAAGTACTGTGGAGTTTGCATGG  
ACAACAGGTGCTGCATCCCCTACAAGTCTAAGACTATCGACGTGTCTTCCAGTGTCTTG  
ATGGGCTTGGCTTCTCCCGCCAGGTCTTATGGA

**FIG.\_27**

SUBSTITUTE SHEET ( rule 26 )

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5' - CAGAATTTGAACTGGGATCCACCTGTCTCTAAAGATGGGTTTCCTCCCATGCTTCCACAC  
TGCTCTCTTGATCAGAAACATACAAGGAGCTGAGAACATGTCTCCACTCCCTGGGTAC  
CTTTGCTGGTTAGAAGCCAACTTGCTGTCCTGTGGGGAGGTACAGCCAATTTCTGTGTTC  
CTCTGAGTTCTGGGGACCGCAGACCTTAGTGTGGTGAAAGTGAGCGTTGGGGGCTGGTGG  
GAGCTGTAGATTCATGCAGATTCTGTTCCCCACACACAGATGCTGTGGGTGAGGTGGAGG  
CATGGCACAGGAACTGCATAGCCTACACAAGCCCCCTGGAGCCCTTGCTCCACCAGCTGCG  
GCCTGGGGGTCTCCACTCGGATCTCCAATGTTAACGCCCAGTGCTGGCCTGAGCAAGAGA  
GCCGCTCTGCAACTTGCGGCCATGCGATGTGGACATCCATACACTCATTAAGGCAGGGA  
AGAAGTGTCTGGCTGTGTACCAGCCAGAGGCATCCATGAACTTCACACTTGCGGGCTGCA  
TCAGCACACGCTCCTATCAACCCAAGTACTGTGGAGTTTGATGGACAATAGGTGCTGCA  
TCCCCTACAAGTCTAAGACTATCGACGTGTCTTCCAGTGTCTTGATGGGCTTGCGTTCT  
CCCGCCAGGTCGTATGGATTAAT

**FIG.\_28**

5' - GTCTGGGCCCAGCTCCCCGAGAGGTGGTCGGATCCTCTGGGCTGCTCGGTGATGCCTG  
TGCCACTGACGTCCAGGCATGAGGTGGTTCTTGCCCTGGACGCTGGCAGCAGTGACAGCA  
GCAGCCGCCAGCACCGTCTTGCCACGGCCCTCTCTCCAGCCCCCTACGACCATGGACTTT  
ACCCAGCTCCACTGGAGGACACCTCCTCACGCCCCCAATTCTGCAAGTGGCCATGTGAG  
TGCCCGCCATCCCCACCCGCTGCCCCGCTGGGGGTGAGCCTCATCACAGATGGCTGTGAG  
TGCTGTAAGATGTGCGCTCAGCAGCTTGGGGACAAGTGCACGGAGGCTGCCATCTGTGAC  
CCCCACCGGGGCTCTACTGTGACTACAGCGGGGACCGCCGAGGTACGCAATAGGAGTG  
TGTGCACGCAGGGAAGAAGTGTCTGGCTGTGTACCAGCCAGAGGCATCCATGAACTTCAC  
ACTTGCGGGCTGCATCAGCACACGCTCCTATCAACCCAAGTACTGTGGAGTTTGATGGA  
CAACAGGTGCTGCATCCCCTACAAGTCTAAGACTATCGACGTGTCTTCCAGTGTCTTGA  
TGGGCTTGCGTTCTCCCGCCAGGTCCTATGGATTAATGCCTGCTTCTGTAACCTGAGCTG  
TAGGAATCCCAATGACATCTTTGCTGACTTGGAATCCTACCCTGACTTCTCAGAAATTGC  
CAACTAGGCAGGCACAAATCTTGGGTCTTGGGGACTAACCCAATGCCTGTGAAGCAGTCA  
GCCCTTATGGCCAATAACTTTTACCAATGAGCCTTAGTTACCCTGATCTGGACCCCTGG  
CCTCCATTTCTGTCTCTAACCATTCAAATGACGCTGATGGTGCTGCTCAGGCCCATGCT  
ATGAGTTTTCTCCTTGATATCATTCAGCATCTACTCTAAAGAAAAATGCCTGTCTCTAGC  
TGTTCTGGACTACACCCAAGCCTGATCCAGCCTTTCCAAGTCACTAGAAGTCTGCTGGA  
TCTTGCCATAAATCCCAAGAAATGGAATCAGGTAGACTTTTAATATCACTAATTTCTTCTT  
TAGATGCCAAACCACAAGACTCTTGGGTCCATTGAGATGAATAGATGGAATTTGGAACA  
ATAGAATAATCTATTATTTGGAGCCTGCCAAGAGGTACTGTAATGGGTAATTCTGACGTC  
AG

**FIG.\_29**



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5' - CAGAACAGCTAGAGACAGGCATTTTTCTTTAGAGTAGATGCTGAATGATATCAAGGAGAA  
AACTCATAGCATGGGCCTGAGCAGCACCATCAGGCGTCATTTGAATGGTTAGAGACAGAA  
ATGGAGGCCAAGGGTCCAGATCAGGGTAACTAAGGCTCATTGGTGAAAAGTTATTGGCCA  
TAAGGGCTGACTGCTTCACAGGCATTGGGTAGTCCCCAAGACCCAAGATTTGTGCCTGC  
CTAGTTGGCAATTTCTGAGAAGTCAGGGTAGGATTCCAAGTCAGCAAAGATGTCATTGGG  
ATTCCCTACAGCTCAGGTTACAGAAGCAGGCATTAATCCATAGGACCTGGCGGGAGAAGCC  
AAGCCCATCAGGACACTGGAAGGACACGTCGATAGTCTTAGACTTGTAGGGGATGCAGCA  
CCTATTGTCCATGCAAACCTCCACAGTACTTGGGTGATAGGAGCGTGTGCTGATGCAGCC  
CGCAAGTGTGAAGTTCATGGATGCCTCTGGCTGGTACACAGCCAGACACTTCTTCCCTGC  
CTTAATGAGTGTATGGATGTCCACATCGCATGGCCGCAAGTTGCAGAGGCGGCTCTCTTG  
CTCAGGCCAGCACTGGGCGTTAACATTGGAGATCCGAGTGGAGACCCCCAGGCCGCAGCT  
GGTGGAGCAAGGGCTCCAGGGGCTTGTGTAGGCTATGCAGTTCCTGTGCCATGCCTCCAC  
CTCACCACAGCATCTGTGTGTGGGGAACAGAATCTGCATGAATCTACAGCTCCCACCAG  
CCCCCAACGCTCACTTTCACCACACTAAGGTCTGCGGTCCCAGAACTCAGAGGAACACA  
GAAATTGGCTGTACCTCCCCACAGGACAGCAAGTTGGCTTCTAACCAGCAAAGGTACCCA  
GGGAGTGGAGGACATGTTCTCAGCTCCTTGTATGTTTCTGATCAAGAGAGGCAGTGTGGA  
AGCATGGGAGGAAACCCATCTTTAGAGACAGGTGGATCCCAGTTCAAATTTCTGCTCTACC  
ACCTACAAGCTGTGTGATCTTAGATAAACCACCCTGGGCTGTCTCCCCATTAGAACAAT  
AACACCTGCCTGTGCGGCTGGCAACACAATAATAAGGGCCTAGATTTTTTACTGAGTATGC  
ATCAATCATCCTTGCTAAGTGCTGGGAATGGGACTTTTTTTTTT

**FIG.\_30**

5' - CCTGATCTGGACCCTTGGCCTCCAATTCTGTCTGTAACCATTCAAATGACGCCTGGTGGT  
GCTGCTCAGGCCCATAGCAAGGTTAGCCTGGTTAAGTCCAAGCTGAATTAGCGGCCGCG  
TCGACAGTAGGAGTGTGTGCACATGCTGTGGGTGAGGTGGAGGCATGGCACAGGAACTGC  
ATAGCCTACACAAGCCCCTGGAGCCCTTGCTCCACCAGCTGCGGCCTGGGGGTCTCCACT  
CGGATCTCCAATGTTAACGCCCAGTGCTGGCCTGAGCAAGAGAGCCGCCTCTGCAACTTG  
CGGCCATGCGATGTGGACATCCATACACTCATTAAGGCAGGGAAGAAGTGTCTGGCTGTG  
TACCAGCCAGAGGCATCCATGAACTTCACACTTGCGGGCTGCATCAGCACACGCTCCTAT  
CAACCCAAGTACTGTGGAGTTTGCATGGACAATAGGTGCTGCATCCCCTACAAGTCTAAG  
ACTATCGACGTGTCCTTCCAGTGTCTGATGGGCTTGGCTTCTCCCGCCAGGTCTTATGG  
ATTAAT

**FIG.\_31**

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5' -GGCCCAGCTCCCCGAGAGGTGGTTCGGATCCTCTGGGCTGCTCGGTGATGCCTGTGCCA  
CTGACGTCCAGGCATGAGGTGGTTCCTGCCCTGGACGCTGGCAGCAGTGACAGCAGCAGC  
CGCCAGCACCGTCCTGGCCACGGCCCTCTCTCCAGCCCCTACGACCATGGACTTTACCCC  
AGCTCCACTGGAGGACACCTCCTCACGCCCCCAATTCTGCAAGTGGCCATGTGAGTGCCC  
GCCATCCCCACCCCGCTGCCCGCTGGGGGTGAGCCTCATCACAGATGGCTGTGAGTGCTG  
TAAGATGTGCGCTCAGCAGCTTGGGGACAACCTGCACGGAGGCTGCCATCTGTGACCCCCA  
CCGGGGCCTCTACTGTGACTACAGCGGGGACCGCCCCGAGAGGTGGTTCGGTGTGGGCTGCG  
TCCTGGATGGGGTGCCTACAACAACGGCCAGTCCTTCCAGCCTAACTGCAAGTACAACCT  
GCACGTGCATCGACGGCGCGGTGGGCTGCACACCACTGTGCCTCCGAGTGCGCCCCCGC  
GTCTCTGGTGCCCCCACCCGCGGCGGTGAGCATACTGGCCACTGCTGTGAGCAGTGGA  
TATGTGAGGACGACGCCAAGAGGCCACGCAAGACCGCACCCCGTGACACAGGAGCCTTCG  
ATGCCAGAAGCGCCCGCTCCCTCAGAGATGTGACAACCAAAATCATCTCCAGACCTTTCC  
AAATACACCCTAGGAGACAAAATTGCTCGGTGGAGAAGCAGTCCTGTGAGGACAGGAGGA  
GGCGTGAGGAAAGCTTTGTCCCCAGCAGCCCCAGGGAAGCAAGGCAGCTCTCCACCAC  
CACCTCCCCAGGAGGGCCACACGAGGGTCACGGGGGGAGCAGGGAGGCGGAAGCTGTCTG  
CCATTGTGTCTGGCCCAGTGACCCTGTTCTGACCGAGCACAAGCGGAGCCCCTGCCTAGC  
CGAGATGCTGTGGGTGAGGTGGAGGCATGGCACAGGAACTGCATAGCCTACACAAGCCCC  
TGGAGCCCTTGCTCCACCAGCTGCGGCCTGGGGGTCTCCACTCGGATCTCCAATGTTAAC  
GCCCAGTGCTGGCCTGAGCAA

**FIG.\_32**

## Sequence Listing

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      55              60              65
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      70              75              80
   Leu Gly Asp Asn Cys Thr Glu Ala Ala Ile Cys Asp Pro His Arg
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      100             105             110
20  Gly Val Cys Ala Gln Val Val Gly Val Gly Cys Val Leu Asp Gly
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   Phe Asp Ala Val Gly Glu Val Glu Ala Trp His Arg Asn Cys Ile
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35  Ala Tyr Thr Ser Pro Trp Ser Pro Cys Ser Thr Ser Cys Gly Leu
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	Ala Trp His Arg	Asn Cys Ile Ala Tyr	Thr Ser Pro Trp Ser Pro
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	Lys Thr Ile Asp	Val Ser Phe Gln Cys	Pro Asp Gly Leu Gly Phe
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	Ser Arg Gln Val	Leu Trp Ile Asn Ala	Cys Phe Cys Asn Leu Ser
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	Glu Gln Glu Ser Arg Leu Cys Asn Leu Arg Pro Cys Asp Val Asp	230	235	240
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	Arg Cys Cys Ile Pro Tyr Lys Ser Lys Thr Ile Asp Val Ser Phe	290	295	300
	Gln Cys Pro Asp Gly Leu Gly Phe Ser Arg Gln Val Leu Trp Ile	305	310	315
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	Ser Ser Thr Gln Ser Tyr Lys Pro Thr Phe Cys Gly Ile Cys Leu		
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	Asp Lys Arg Cys Cys Ile Pro Asn Lys Ser Lys Met Ile Thr Ile		
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35	Gly Leu Ala Gln Phe Cys Cys Arg Val Gln Gly Thr Gly Pro Leu		
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	Asp Thr Thr Pro Glu Gly Arg Pro Gly Glu Val Ser Asp Ala Pro		
	50	55	60
40	Gln Arg Lys Gln Phe Cys His Trp Pro Cys Lys Cys Pro Gln Gln		
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&lt;211&gt; 339

30 &lt;212&gt; PRT

&lt;213&gt; Human

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	260 265 270	
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 50 55 60  
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 65 70 75  
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 Pro Ala Tyr Arg Asn Leu Pro Leu Ile Trp Lys Lys Lys Cys Leu  
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 40 Val Gln Ala Thr Lys Trp Thr Pro Cys Ser Arg Thr Cys Gly Met  
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	Thr	Phe	Gln	Leu	Ser	Lys	Ala	Glu	Lys	Phe	Val	Phe	Ser	Gly	Cys
					275					280				285	
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5	Glu Pro Cys Asp Gln Leu His Val Cys Asp Ala Ser Gln Gly Leu		
	65	70	75
	Val Cys Gln Pro Gly Ala Gly Pro Gly Gly Arg Gly Ala Leu Cys		
	80	85	90
	Leu Leu Ala Glu Asp Asp Ser Ser Cys Glu Val Asn Gly Arg Leu		
	95	100	105
10	Tyr Arg Glu Gly Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys		
	110	115	120
	Arg Cys Glu Asp Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu		
	125	130	135
15	Asp Val Arg Leu Pro Ser Trp Asp Cys Pro His Pro Arg Arg Val		
	140	145	150
	Glu Val Leu Gly Lys Cys Cys Pro Glu Trp Val Cys Gly Gln Gly		
	155	160	165
	Gly Gly Leu Gly Thr Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe		
	170	175	180
20	Ser Gly Leu Val Ser Ser Leu Pro Pro Gly Val Pro Cys Pro Glu		
	185	190	195
	Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly		
	200	205	210
25	Met Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Arg Leu Glu		
	215	220	225
	Thr Gln Arg Arg Leu Cys Leu Ser Arg Pro Cys Pro Pro Ser Arg		
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	Gly Arg Ser Pro Gln Asn Ser Ala Phe		
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40	Pro Trp Pro Pro Pro Arg Cys Pro Leu Gly Val Pro Leu Val Leu		
	35	40	45

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 Gln Pro Gly Ala Gly Pro Gly Gly Arg Gly Ala Leu Cys Leu Leu  
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 Glu Gly Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys  
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 10 Glu Asp Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val  
 125 130 135  
 Arg Leu Pro Ser Trp Asp Cys Pro His Pro Arg Arg Val Glu Val  
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 155 160 165  
 15 Leu Gly Thr Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly  
 170 175 180  
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 185 190 195  
 20 Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala  
 200 205 210  
 Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln  
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 40 Asp Gln Leu His Val Cys Asp Ala Ser Gln Gly Leu Val Cys Gln  
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	Glu Asp Asp Ser	Ser Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu	
	95	100	105
5	Gly Glu Thr Phe	Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu	
	110	115	120
	Asp Gly Gly Phe	Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg	
	125	130	135
10	Leu Pro Ser Trp	Asp Cys Pro His Pro Arg Arg Val Glu Val Leu	
	140	145	150
	Gly Lys Cys Cys	Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu	
	155	160	165
	Gly Thr Gln Pro	Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu	
	170	175	180
15	Val Ser Ser Leu	Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr	
	185	190	195
	Ala Trp Gly Pro	Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr	
	200	205	210
20	Arg Val Ser Asn	Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg	
	215	220	225
	Arg Leu Cys Leu	Ser Arg Pro Cys Pro Pro Ser Arg Gly Arg Ser	
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35	Pro Pro Arg Cys	Pro Leu Gly Val Pro Leu Val Leu Asp Gly Cys	
	35	40	45
	Gly Cys Cys Arg	Val Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp	
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	Gln Leu His Val	Cys Asp Ala Ser Gln Gly Leu Val Cys Gln Pro	
	65	70	75
40	Gly Ala Gly Pro	Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala Glu	
	80	85	90



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	Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp	110	115	120
5	Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu	125	130	135
	Pro Ser Trp Asp Cys Pro His Pro Arg Arg Val Glu Val Leu Gly	140	145	150
10	Lys Cys Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly	155	160	165
	Thr Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val	170	175	180
	Ser Ser Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala	185	190	195
15	Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg	200	205	210
	Val Ser Asn Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg	215	220	225
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	Pro Arg Cys Pro Leu Gly Val Pro Leu Val Leu Asp Gly Cys Gly	35	40	45
35	Cys Cys Arg Val Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln	50	55	60
	Leu His Val Cys Asp Ala Ser Gln Gly Leu Val Cys Gln Pro Gly	65	70	75
	Ala Gly Pro Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala Glu Asp	80	85	90
40	Asp Ser Ser Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu Gly Glu	95	100	105

Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly  
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 Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu Pro  
 125 130 135  
 5 Ser Trp Asp Cys Pro His Pro Arg Arg Val Glu Val Leu Gly Lys  
 140 145 150  
 Cys Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr  
 155 160 165  
 10 Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser  
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 Ser Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp  
 185 190 195  
 Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val  
 200 205 210  
 15 Ser Asn Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg Leu  
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 Cys Leu Ser Arg Pro Cys Pro Pro Ser Arg Gly Arg Ser Pro Gln  
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 Cys Arg Val Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln Leu  
 50 55 60  
 35 His Val Cys Asp Ala Ser Gln Gly Leu Val Cys Gln Pro Gly Ala  
 65 70 75  
 Gly Pro Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala Glu Asp Asp  
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 Ser Ser Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu Gly Glu Thr  
 95 100 105  
 40 Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly  
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 5 Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr Gln  
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 Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser Ser  
 170 175 180  
 10 Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly  
 185 190 195  
 Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser  
 200 205 210  
 Asn Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg Leu Cys  
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 30 Arg Val Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln Leu His  
 50 55 60  
 Val Cys Asp Ala Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly  
 65 70 75  
 35 Pro Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala Glu Asp Asp Ser  
 80 85 90  
 Ser Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu Gly Glu Thr Phe  
 95 100 105  
 Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly Phe  
 110 115 120  
 40 Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp  
 125 130 135

	Asp Cys Pro His Pro Arg Arg Val Glu Val Leu Gly Lys Cys Cys	140	145	150
	Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr Gln Pro	155	160	165
5	Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser Ser Leu	170	175	180
	Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly Pro	185	190	195
10	Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser Asn	200	205	210
	Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg Leu Cys Leu	215	220	225
	Ser Arg Pro Cys Pro Pro Ser Arg Gly Arg Ser Pro Gln Asn Ser	230	235	240
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	Pro Leu Gly Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Arg	35	40	45
	Val Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln Leu His Val	50	55	60
30	Cys Asp Ala Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro	65	70	75
	Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala Glu Asp Asp Ser Ser	80	85	90
35	Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu Gly Glu Thr Phe Gln	95	100	105
	Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly Phe Thr	110	115	120
	Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp	125	130	135
40	Cys Pro His Pro Arg Arg Val Glu Val Leu Gly Lys Cys Cys Pro	140	145	150

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 Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser Ser Leu Pro  
 170 175 180  
 5 Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly Pro Cys  
 185 190 195  
 Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser Asn Gln  
 200 205 210  
 10 Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg Leu Cys Leu Ser  
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 Cys Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln Leu His Val Cys  
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 95 100 105  
 35 His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly Phe Thr Cys  
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 140 145 150  
 40 Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr Gln Pro Leu Pro  
 155 160 165

Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser Ser Leu Pro Pro  
170 175 180

Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly Pro Cys Ser  
185 190 195

5 Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser Asn Gln Asn  
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Ala Arg Arg Leu Gly Glu Pro Cys Asp Gln Leu His Val Cys Asp  
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25 Ala Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Gly Gly  
65 70 75

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Val Asn Gly Arg Leu Tyr Arg Glu Gly Glu Thr Phe Gln Pro His  
95 100 105

30 Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly Phe Thr Cys Val  
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Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro  
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35 His Pro Arg Arg Val Glu Val Leu Gly Lys Cys Cys Pro Glu Trp  
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Val Cys Gly Gln Gly Gly Gly Leu Gly Thr Gln Pro Leu Pro Ala  
155 160 165

Gln Gly Pro Gln Phe Ser Gly Leu Val Ser Ser Leu Pro Pro Gly  
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40 Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr  
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Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser Asn Gln Asn Arg  
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15 Arg Leu Gly Glu Pro Cys Asp Gln Leu His Val Cys Asp Ala Ser  
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Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Gly Gly Arg Gly  
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Gly Gln Gly Gly Gly Leu Gly Thr Gln Pro Leu Pro Ala Gln Gly  
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Cys Pro Glu Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys  
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	Leu Val Cys Gln	Pro Gly Ala Gly	Pro Gly Gly Arg	Gly Ala Leu
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	Leu Tyr Arg Glu	Gly Glu Thr Phe Gln	Pro His Cys Ser	Ile Arg
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	Glu Asp Val Arg	Leu Pro Ser Trp Asp	Cys Pro His Pro	Arg Arg
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	Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Arg Arg Leu Gly	35	40	45
	Glu Pro Cys Asp Gln Leu His Val Cys Asp Ala Ser Gln Gly Leu	50	55	60
5	Val Cys Gln Pro Gly Ala Gly Pro Gly Gly Arg Gly Ala Leu Cys	65	70	75
	Leu Leu Ala Glu Asp Asp Ser Ser Cys Glu Val Asn Gly Arg Leu	80	85	90
10	Tyr Arg Glu Gly Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys	95	100	105
	Arg Cys Glu Asp Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu	110	115	120
	Asp Val Arg Leu Pro Ser Trp Asp Cys Pro His Pro Arg Arg Val	125	130	135
15	Glu Val Leu Gly Lys Cys Cys Pro Glu Trp Val Cys Gly Gln Gly	140	145	150
	Gly Gly Leu Gly Thr Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe	155	160	165
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	Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly	185	190	195
	Met Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Arg Leu Glu	200	205	210
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40	Pro Cys Asp Gln Leu His Val Cys Asp Ala Ser Gln Gly Leu Val	50	55	60

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	Glu Gly Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys	95	100	105
	Glu Asp Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val	110	115	120
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	Leu Gly Lys Cys Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly	140	145	150
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	Leu Val Ser Ser Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser	170	175	180
	Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala	185	190	195
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	Asp Gln Leu His Val Cys Asp Ala Ser Gln Gly Leu Val Cys Gln	50	55	60
35	Pro Gly Ala Gly Pro Gly Gly Arg Gly Ala Leu Cys Leu Leu Ala	65	70	75
	Glu Asp Asp Ser Ser Cys Glu Val Asn Gly Arg Leu Tyr Arg Glu	80	85	90
	Gly Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu	95	100	105
40	Asp Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg	110	115	120

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 5 Gly Thr Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu  
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 185 190 195  
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 65 70 75  
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 80 85 90  
 35 Glu Thr Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp  
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 Gly Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu  
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 140 145 150

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 Ser Ser Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala  
 170 175 180  
 5 Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg  
 185 190 195  
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 65 70 75  
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 Gly Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu Pro  
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 Cys Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr  
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 Gln Pro Leu Pro Ala Gln Gly Pro Gln Phe Ser Gly Leu Val Ser  
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 80 85 90  
 Phe Gln Pro His Cys Ser Ile Arg Cys Arg Cys Glu Asp Gly Gly  
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 Phe Thr Cys Val Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser  
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 Cys Pro Glu Trp Val Cys Gly Gln Gly Gly Gly Leu Gly Thr Gln  
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 Leu Pro Pro Gly Val Pro Cys Pro Glu Trp Ser Thr Ala Trp Gly  
 170 175 180  
 Pro Cys Ser Thr Thr Cys Gly Leu Gly Met Ala Thr Arg Val Ser  
 185 190 195  
 40 Asn Gln Asn Arg Phe Cys Arg Leu Glu Thr Gln Arg Arg Leu Cys  
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Ser Ala Phe
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15 Cys Pro Trp Thr Pro Pro Gln Cys Pro Pro Gly Val Pro Leu Val
    35                      40                      45

    Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Arg Arg Leu Gly
      50                      55                      60

    Glu Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly Leu
      65                      70                      75

20 Val Cys Gln Pro Glu Ala Gly Pro Ser Gly Arg Gly Ala Val Cys
    80                      85                      90

    Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg
      95                      100                     105

    Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys
    110                     115                     120

25 Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu
    125                     130                     135

    Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile
    140                     145                     150

30 Gln Val Pro Gly Arg Cys Cys Pro Glu Trp Val Cys Asp Gln Ala
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    Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln Gly His Gln
    170                     175                     180

    Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro
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35 Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu
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    Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Gln Leu
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 Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe  
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 Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu  
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 10 His Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro  
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 Gly Ala Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu  
 80 85 90  
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 Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp  
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 Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu  
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 20 Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly  
 140 145 150  
 Arg Cys Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro  
 155 160 165  
 25 Ala Ile Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu  
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 Val Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr  
 185 190 195  
 Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr  
 200 205 210  
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	Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly	65	70	75
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	Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu	95	100	105
15	Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly	110	115	120
	Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro	125	130	135
	Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg	140	145	150
20	Cys Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala	155	160	165
	Ile Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val	170	175	180
25	Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala	185	190	195
	Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg	200	205	210
	Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg	215	220	225
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	His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala		70	75
		65		
10	Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu Asp Asp		85	90
		80		
	Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr		100	105
		95		
	Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly Gly		115	120
		110		
15	Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser		130	135
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	Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg Cys		145	150
		140		
20	Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala Ile		160	165
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	Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr		175	180
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	Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp		190	195
		185		
25	Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg Val		205	210
		200		
	Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu		220	225
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15	Asp	Cys	Pro	Arg	Pro	Arg	Arg	Ile	Gln	Val	Pro	Gly	Arg	Cys	Cys	
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	Pro	Glu	Trp	Val	Cys	Asp	Gln	Ala	Val	Met	Gln	Pro	Ala	Ile	Gln	
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	Val Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val	
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	Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro	
	65	70 75
5	Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu Asp Asp Gly Ser	
	80	85 90
	Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys	
	95	100 105
10	Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr	
	110	115 120
	Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp	
	125	130 135
	Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg Cys Cys Pro	
	140	145 150
15	Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala Ile Gln Pro	
	155	160 165
	Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala	
	170	175 180
20	Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro	
	185	190 195
	Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg Val Ser Asn	
	200	205 210
	Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu Cys Leu	
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25	Ser Arg Pro Cys Leu Ala Ser Arg Ser His Gly Ser Trp Asn Ser	
	230	235 240
	Ala Phe	
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	20 25 30	
	Pro Gly Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val	
	35 40 45	
40	Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys	
	50 55 60	

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      Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Ser
                        65                      70                      75

      Gly Arg Gly Ala Val Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys
                        80                      85                      90

5     Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro
                        95                      100                     105

      Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys
                        110                     115                     120

10    Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys
                        125                     130                     135

      Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg Cys Cys Pro Glu
                        140                     145                     150

      Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala Ile Gln Pro Ser
                        155                     160                     165

15    Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser
                        170                     175                     180

      Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys
                        185                     190                     195

20    Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg Val Ser Asn Gln
                        200                     205                     210

      Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu Cys Leu Ser
                        215                     220                     225

      Arg Pro Cys Leu Ala Ser Arg Ser His Gly Ser Trp Asn Ser Ala
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25    Phe
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35    20                      25                      30

      Gly Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys
          35                      40                      45

      Ala Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp
          50                      55                      60

40    Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Ser Gly
          65                      70                      75

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	Arg Gly Ala Val Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu	80	85	90
	Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn	95	100	105
5	Cys Arg Val Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu	110	115	120
	Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro	125	130	135
10	Arg Pro Arg Arg Ile Gln Val Pro Gly Arg Cys Cys Pro Glu Trp	140	145	150
	Val Cys Asp Gln Ala Val Met Gln Pro Ala Ile Gln Pro Ser Ser	155	160	165
	Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala	170	175	180
15	Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser	185	190	195
	Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg Val Ser Asn Gln Asn	200	205	210
20	Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu Cys Leu Ser Arg	215	220	225
	Pro Cys Leu Ala Ser Arg Ser His Gly Ser Trp Asn Ser Ala Phe	230	235	240
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	Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala	35	40	45
35	Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp Pro	50	55	60
	Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg	65	70	75
	Gly Ala Val Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val	80	85	90
40	Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys	95	100	105

	Arg Val Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro	110	115	120
	Leu Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg	125	130	135
5	Pro Arg Arg Ile Gln Val Pro Gly Arg Cys Cys Pro Glu Trp Val	140	145	150
	Cys Asp Gln Ala Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala	155	160	165
10	Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp	170	175	180
	Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr	185	190	195
	Thr Cys Gly Leu Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg	200	205	210
15	Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu Cys Leu Ser Arg Pro	215	220	225
	Cys Leu Ala Ser Arg Ser His Gly Ser Trp Asn Ser Ala Phe	230	235	239
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	Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Arg	35	40	45
30	Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp Pro Ser	50	55	60
	Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly	65	70	75
35	Ala Val Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn	80	85	90
	Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg	95	100	105
	Val Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu	110	115	120
40	Cys Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro	125	130	135

	Arg Arg Ile Gln Val Pro Gly Arg Cys Cys Pro Glu Trp val Cys	140	145	150
	Asp Gln Ala Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln	155	160	165
5	Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly	170	175	180
	Pro Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr	185	190	195
10	Cys Gly Leu Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe	200	205	210
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	Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln	50	55	60
	Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly Ala	65	70	75
30	Val Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly	80	85	90
	Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val	95	100	105
35	Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys	110	115	120
	Ser Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Arg	125	130	135
	Arg Ile Gln Val Pro Gly Arg Cys Cys Pro Glu Trp Val Cys Asp	140	145	150
40	Gln Ala Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln Gly	155	160	165

His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly Pro  
 170 175 180

Cys Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys  
 185 190 195

5 Gly Leu Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys  
 200 205 210

Gln Leu Glu Ile Gln Arg Arg Leu Cys Leu Ser Arg Pro Cys Leu  
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 35 40 45

Gly Glu Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly  
 50 55 60

25 Leu Val Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly Ala Val  
 65 70 75

Cys Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg  
 80 85 90

Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val Leu  
 95 100 105

30 Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser  
 110 115 120

Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg  
 125 130 135

35 Ile Gln Val Pro Gly Arg Cys Cys Pro Glu Trp Val Cys Asp Gln  
 140 145 150

Ala Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln Gly His  
 155 160 165

Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly Pro Cys  
 170 175 180

40 Pro Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly  
 185 190 195

Leu Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Gln  
 200 205 210  
 Leu Glu Ile Gln Arg Arg Leu Cys Leu Ser Arg Pro Cys Leu Ala  
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 35 40 45  
 Glu Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly Leu  
 50 55 60  
 20 Val Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly Ala Val Cys  
 65 70 75  
 Leu Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg  
 80 85 90  
 25 Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys  
 95 100 105  
 Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu  
 110 115 120  
 Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile  
 125 130 135  
 30 Gln Val Pro Gly Arg Cys Cys Pro Glu Trp Val Cys Asp Gln Ala  
 140 145 150  
 Val Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln Gly His Gln  
 155 160 165  
 35 Leu Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro  
 170 175 180  
 Asn Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu  
 185 190 195  
 Gly Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Gln Leu  
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 40 Glu Ile Gln Arg Arg Leu Cys Leu Ser Arg Pro Cys Leu Ala Ser  
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    Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Arg Arg Leu Gly Glu
      35              40              45

15  Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly Leu Val
      50              55              60

    Cys Gln Pro Gly Ala Gly Pro Ser Gly Arg Gly Ala Val Cys Leu
      65              70              75

    Phe Glu Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr
      80              85              90

20  Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg
      95              100             105

    Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp
      110             115             120

25  Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln
      125             130             135

    Val Pro Gly Arg Cys Cys Pro Glu Trp Val Cys Asp Gln Ala Val
      140             145             150

    Met Gln Pro Ala Ile Gln Pro Ser Ser Ala Gln Gly His Gln Leu
      155             160             165

30  Ser Ala Leu Val Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn
      170             175             180

    Trp Ser Thr Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly
      185             190             195

    Ile Ala Thr Arg Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu
      200             205             210

35  Ile Gln Arg Arg Leu Cys Leu Ser Arg Pro Cys Leu Ala Ser Arg
      215             220             225

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-103-

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		35	40	45
	Asp His Leu His	Val Cys Asp Pro Ser	Gln Gly Leu Val Cys Gln	
		50	55	60
	Pro Gly Ala Gly	Pro Ser Gly Arg Gly	Ala Val Cys Leu Phe Glu	
		65	70	75
10	Glu Asp Asp Gly	Ser Cys Glu Val Asn	Gly Arg Arg Tyr Leu Asp	
		80	85	90
	Gly Glu Thr Phe	Lys Pro Asn Cys Arg	Val Leu Cys Arg Cys Asp	
		95	100	105
15	Asp Gly Gly Phe	Thr Cys Leu Pro Leu	Cys Ser Glu Asp Val Arg	
		110	115	120
	Leu Pro Ser Trp	Asp Cys Pro Arg Pro	Arg Arg Ile Gln Val Pro	
		125	130	135
	Gly Arg Cys Cys	Pro Glu Trp Val Cys	Asp Gln Ala Val Met Gln	
		140	145	150
20	Pro Ala Ile Gln	Pro Ser Ser Ala Gln	Gly His Gln Leu Ser Ala	
		155	160	165
	Leu Val Thr Pro	Ala Ser Ala Asp Gly	Pro Cys Pro Asn Trp Ser	
		170	175	180
25	Thr Ala Trp Gly	Pro Cys Ser Thr Thr	Cys Gly Leu Gly Ile Ala	
		185	190	195
	Thr Arg Val Ser	Asn Gln Asn Arg Phe	Cys Gln Leu Glu Ile Gln	
		200	205	210
	Arg Arg Leu Cys	Leu Ser Arg Pro Cys	Leu Ala Ser Arg Ser His	
		215	220	225
30	Gly Ser Trp Asn	Ser Ala Phe		
		230	232	
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		20	25	30

	Gly Cys Cys Ala Val Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp	35	40	45
	His Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro	50	55	60
5	Gly Ala Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu	65	70	75
	Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly	80	85	90
10	Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp	95	100	105
	Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu	110	115	120
	Pro Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly	125	130	135
15	Arg Cys Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro	140	145	150
	Ala Ile Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu	155	160	165
20	Val Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr	170	175	180
	Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr	185	190	195
	Arg Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg	200	205	210
25	Arg Leu Cys Leu Ser Arg Pro Cys Leu Ala Ser Arg Ser His Gly	215	220	225
	Ser Trp Asn Ser Ala Phe	230	231	
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	Cys Cys Arg Val Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp His	35	40	45
40	Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly	50	55	60

Ala Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu Asp  
65 70 75

Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu  
80 85 90

5 Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly  
95 100 105

Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro  
110 115 120

10 Ser Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg  
125 130 135

Cys Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala  
140 145 150

Ile Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val  
155 160 165

15 Thr Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala  
170 175 180

Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg  
185 190 195

20 Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg  
200 205 210

Leu Cys Leu Ser Arg Pro Cys Leu Ala Ser Arg Ser His Gly Ser  
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Trp Asn Ser Ala Phe  
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35 40 45

His Val Cys Asp Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala  
50 55 60

Gly Pro Ser Gly Arg Gly Ala Val Cys Leu Phe Glu Glu Asp Asp  
65 70 75

40 Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly Glu Thr  
80 85 90

Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly Gly  
                                     95                                    100                                    105  
  
 Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser  
                                     110                                    115                                    120  
  
 5 Trp Asp Cys Pro Arg Pro Arg Arg Ile Gln Val Pro Gly Arg Cys  
                                     125                                    130                                    135  
  
 Cys Pro Glu Trp Val Cys Asp Gln Ala Val Met Gln Pro Ala Ile  
                                     140                                    145                                    150  
  
 10 Gln Pro Ser Ser Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr  
                                     155                                    160                                    165  
  
 Pro Ala Ser Ala Asp Gly Pro Cys Pro Asn Trp Ser Thr Ala Trp  
                                     170                                    175                                    180  
  
 Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg Val  
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                                     200                                    205                                    210  
  
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gcacctaccg acccaagtac 70

35 <210> 114  
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<400> 122  
acaatacagc cctttgtgtg ggtcaca 27

<210> 123  
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15 <400> 125  
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<210> 127  
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35 <400> 127  
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45 <400> 128  
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5 <220>  
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<400> 129  
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<210> 130  
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<210> 131  
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35 <220>  
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45 <210> 138

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<400> 140  
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40 <210> 142  
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<220>  
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45 <211> 48

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<210> 155  
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<400> 155  
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<210> 156  
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<222> 1-48  
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ctatgaaatt aaccctcact aaaggagct gtcgcaaggc tgaatgta 48

# INTERNATIONAL SEARCH REPORT

Internat Application No  
PCT/US 98/22991

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/12 C07K14/47 C12N15/62 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE EMBL - EMBEST1 Entry Aa592984, Acc.no. AA592984, 24 September 1997 STRAUSBERG, R.: "nn03e01.s1 NCI_CGAP_Pr4.1 Homo sapiens cDNA clone IMAGE:1076664 similar to TR:G984956 G984956 CONNECTIVE TISSUE GROWTH FACTOR" XP002094092 see the whole document ----- -/--</p>	<p>47, 49-54, 57-60, 62-68, 71,72, 79,81, 83,85,86</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

19 February 1999

Date of mailing of the international search report

05/03/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

## INTERNATIONAL SEARCH REPORT

Interr 1st Application No

PCT/US 98/22991

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE EMBL - EMHUM1 Entry Hs14217, Acc.no. Z99289, 17 September 1997 TUBBY B.: "Homo sapiens DNA sequence from PAC 142L7 on chromosome 6q21. Contains a...Connective tissue growth factor (NOV, GIG) LIKE gene,..." XP002094093 nt. 12398-12855</p> <p>----</p>	<p>47, 49-54, 57-60, 62-68, 71,72, 79,81, 83,85,86</p>
Y	<p>OEMAR, B.S. ET AL.: "Connective tissue growth factor - friend or foe?" ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, vol. 17, no. 8, August 1997, pages 1483-9, XP002094090 cited in the application see the whole document</p> <p>----</p>	<p>47, 49-54, 57-60, 62-68, 71,72, 79,81, 83,85,86</p>
A	<p>DATABASE EMBL - EMBEST10 Entry HS01627, Acc.no T55016, 28 February 1995 HILLIER, L. ET AL.: "yb42e03.r1 Homo sapiens cDNA clone 73852 5'" XP002094094 see abstract</p> <p>----</p>	<p>1,13</p>
A	<p>DATABASE EMBL - EMBEST18 Entry Hszz82583, Acc.no. AA377456, 18 April 1997 ADAMS, M.D. ET AL.: "EST90040 Synovial membrane Homo sapiens cDNA 5' end." XP002094095 see the whole document -&amp; ADAMS, M.D. ET AL.: "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence" NATURE, vol. 377, 1995, pages 3-17, XP002042918 see the whole document</p> <p>----</p>	<p>26, 28-30,35</p>
P,X	<p>HASHIMOTO, Y. ET AL.: "Expression of the Elm1 gene, a novel gene of the CCN (Connective tissue growth factor, Cyr61/Cef10, and neuroblastoma overexpressed gene) family, suppresses in vivo tumor growth and metastasis of K-1735 murine melanoma cells." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 187, no. 3, 2 February 1998, pages 289-96, XP002094091 cited in the application see whole document, particularly fig. 1</p> <p>----</p> <p style="text-align: center;">-/--</p>	<p>1,9,10, 12-19, 24,25</p>

## INTERNATIONAL SEARCH REPORT

Interr 1st Application No

PCT/US 98/22991

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 21236 A (HUMAN GENOME SCIENCES INC ;CHOPRA ARVIND (US); EBNER REINHARD (US)) 22 May 1998  see whole document, particularly seq. 1 and 2, example 1, claim 18 ----	26, 28-30, 35-46, 77,78, 83,85, 86,88, 92,111
P,X	ZHANG, R. ET AL.: "Identification of rCop-1, a new member of the CCN protein family, as a negative regulator for cell transformation" MOLECULAR AND CELLULAR BIOLOGY, vol. 18, no. 10, October 1998, pages 6131-41, XP002094139 cited in the application see whole document, particularly fig.2 and p.6132, left-hand column, second full paragraph -----	26, 30-32, 34,35, 37-40, 42-46,92

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/22991

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 2, 11, 27, 48, 61  
because they relate to subject matter not required to be searched by this Authority, namely:  
See Further Information sheet PCT/ISA/210
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
See Further Information sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98 /22991

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 89-91 and 101, and claims 102-104 and 107 in as far as they relate to use in vivo, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Claims Nos.: 2,11,27,48,61

Claims 2,11,27,48, and 61, referring to biological activities of the claimed WISP proteins, could not be searched due to lack of support of such activities in the discription (Article 6, PCT).

Claims 74,76,78,80,82,105,108,111,114, and 115 referring to antagonists of the claimed WISP polypeptides and/or inhibitors of expression of the claimed WISP genes, could not be searched to completion due to insufficient disclosure of the compounds in the discription (Article 6, PCT).

## INTERNATIONAL SEARCH REPORT

### Information on patent family members

Internal Application No	
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PCT/US 98/22991

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9821236 A	22-05-1998	AU 7610396 A	03-06-1998